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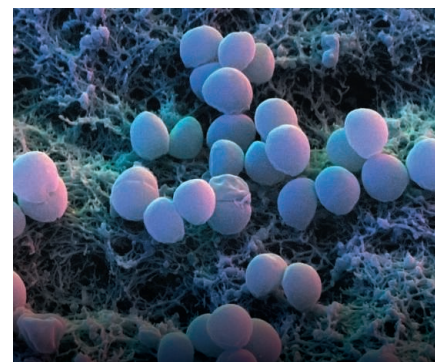
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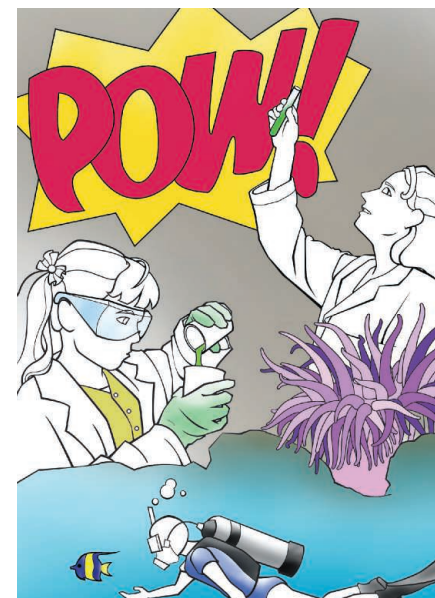
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A domestic cat (*Felis catus*) laps milk by lowering its tongue to touch the liquid surface and then rapidly pulling it up, creating a liquid column, the top of which the animal captures in its mouth before gravity draws it down. Measurements of lapping frequency in domestic and wild cats suggest that this mechanism is conserved among felines and that lapping frequency is tuned to maximize the ingested volume per lap. See page 1231.

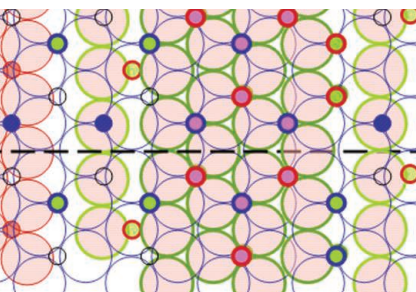
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10.1126/science.1198366

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10.1126/science.1195591

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Group argues that ancient reptiles flew just fine, despite their massive weight.

Evolutionary Relationships Hold, Even in Our Guts

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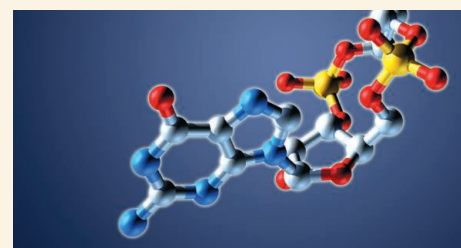
www.sciencetranslationalmedicine.org

Integrating Medicine and Science

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C. Szabo

Endogenous gaseous mediators offer therapeutic opportunities, as well as unexpected challenges for translational science.



SCIENCE SIGNALING

Cyclic-di-GMP, a bacterial second messenger.

RESEARCH ARTICLE: Adducin- and Ouabain-Related Gene Variants Predict the Antihypertensive Activity of Rostafuroxin. Part 1—Experimental Studies

M. Ferrandi et al.

RESEARCH ARTICLE: Adducin- and Ouabain-Related Gene Variants Predict the Antihypertensive Activity of Rostafuroxin. Part 2—Clinical Studies

C. Lanzani et al.

Genetic variants that affect Na,K-ATPase interactions predict the blood pressure response to an antihypertensive drug.

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Science Policy News and Analysis

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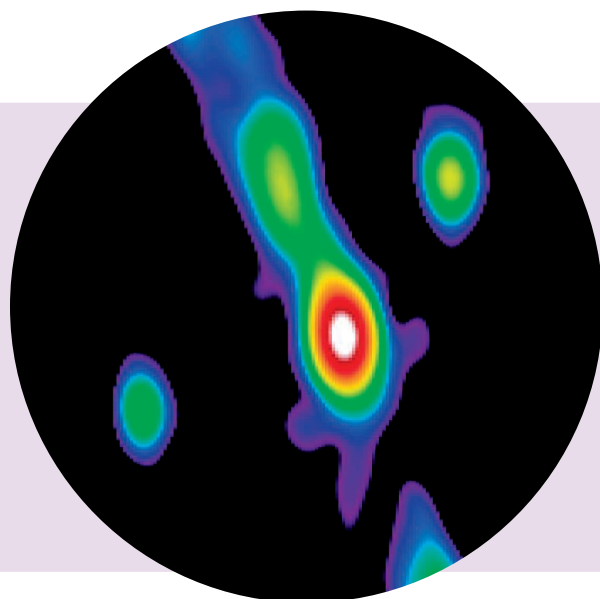
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ADVANCING SCIENCE. SERVING SOCIETY

Stellar Jets >>

Supersonic jets of energized charged particles are a common phenomenon in astrophysics, emanating from sources that range widely in mass: from brown dwarfs to super massive black holes in the centers of galaxies. Carrasco-González *et al.* (p. 1209; see the Perspective by Ray) present observations, of a jet emanating from a young stellar object, which show that the jet is magnetized and has characteristics that are similar to those of jets found in much larger and more massive systems. The results support the idea that all astrophysical jets are launched and collimated through the same basic mechanism, involving launching of material along magnetic field lines.



Macromolecular Message Translation

The ribosome is a macromolecular machine that translates the sequence of messenger RNA into proteins in all living cells. Structures of pro-

karyotic ribosomes have supplied insight into the conserved features of such protein synthesis; however, eukaryotic translation has additional levels of complexity. Ben-Shem *et al.* (p. 1203) have determined the crystal structure of the yeast 80S ribosome at 4.15 angstrom resolution. The ribosome is in a ratcheted conformation, which is a state that is an intermediate in the translocation of messenger RNA and transfer RNA. The crystal structure provides the molecular underpinning for existing biochemical and genetic data and will inform the design of functional experiments.

Dressing-Up Diamond Defects

The spin states of nitrogen vacancy defects in diamond are being explored as information carriers and memories in quantum information systems. Their long lifetimes, fast manipulation rates, and the ability to couple them to adjacent electronic and nuclear spins provide the necessary properties for implementation in solid-state quantum networks. To date, however, the readout of the spin state via photoluminescence, either directly or indirectly, results in the destruction of the spin state. Buckley *et al.* (p. 1212, published

online 14 October; see the Perspective by Milburn) have formed a light-matter hybrid state in which the spin interacts with laser light to form a polariton state. This hybrid state can be optically probed to produce a nondestructive measurement and manipulation technique for the spin state of the nitrogen-vacancy center.

A Little Help from Hydrogen

Biomass may one day displace petroleum as the chemical industry's primary feedstock. Currently, though, the primary hurdle for incorporating plant-derived material into existing process feeds is the high proportion of oxygen in its molecular frameworks. Rapid heating of the biomass followed by high-temperature treatment with zeolite catalysts can yield tractable quantities of useful commodity compounds such as ethylene and benzene, but much of the carbon is wasted in the process—diverted either toward gaseous CO and CO₂, or solid coke. Vispute *et al.* (p. 1222) show that an intermediate step, in which hydrogen is catalytically incorporated into the heated material prior to zeolite treatment, can substantially raise the yield of useful products by reducing susceptibility to coking.

How Mammals Grew in Size

Mammals diversified greatly after the end-Cretaceous extinction, which eliminated the dominant land animals (dinosaurs). Smith *et al.* (p. 1216) examined how the maximum size of mammals increased during their radiation in each continent. Overall, mammal size increased rapidly, then leveled off after about 25 million

years. This pattern holds true on most of the continents—even though data are sparse for South America—and implies that mammals grew to fill available niches before other environmental and biological limits took hold.

Lap Cats

We all know that domestic cats lap milk, but perhaps fewer of us have thought about how they do this. Reis *et al.* (p. 1231, published online 11 November; see the cover) have discovered that cats curl their tongues so that the top surface touches the water. Then, by lifting their tongues rapidly, a column of liquid grows by inertia until gravity induces its breakage and the cats close their jaws to capture the liquid. Lapping frequency is tuned to maximize the volume ingested, depending on the animal's mass; a relationship that holds as true for tabby cats as it does for lions.

Writing to Close Gaps

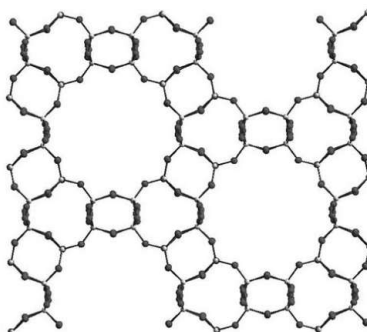
Some have questioned whether findings in the laboratory obtained under controlled conditions and limited contexts bear any relevance to behavior in real-world environments in which ordinary people cope with real-life challenges. Recent studies have shown a replicable and long-term effect of a brief writing exercise on the academic performance of African-American seventh graders in an inner-city public school. Miyake *et al.* (p. 1234) extended this approach to show that a similar kind of writing exercise can help to reduce the gender gap observed in the performance of female students in an undergraduate physics class, where performance is measured not only via course grades and exam scores, but also on a standardized test.

Lynx Vision

Early in development, correct visual experiences during the so-called “critical period” build the foundations for visual function in adulthood. Hence, when one eye is not working together with the other, an adult may be left with imperfect vision. The plasticity characteristic of the critical period does not persist into adulthood, and later readjustments to visual function may not be fully successful. **Morishita *et al.*** (p. 1238, published online 11 November; see the Perspective by **Higley and Strittmatter**) have identified a gene in mice called *Lynx1*, which shows increased expression after the critical period. The Lynx1 protein binds to and reduces the sensitivity of acetylcholine receptors, but if mice were treated to enhanced cholinergic signaling, their adult visual plasticity was improved and if mice lacked the *Lynx1* gene altogether, they were able to recover visual function even in adulthood.

Routes to Rare Zeolites

Zeolites are microporous crystalline solids with well-defined structures. Although many naturally occurring ones have been obtained in laboratory synthesis, some have remained elusive. One of these, boggsite, is of interest for catalytic reactions because it has large channels defined by rings of 10 or 12 atoms that intersect within its crystalline lattice. **Simancas *et al.*** (p. 1219) report the synthesis of boggsite by using phosphazenes as the organic groups that directed the formation of rings during synthesis. These reagents can be readily modified—a feature that should allow greater flexibility in synthesis routes.



By a Whisker

Every student learns that the sensory cortex is used for processing sensation and the motor cortex is used for perceiving movement. However, in the real world, this may not always be so neatly arranged. **Matyas *et al.*** (p. 1240) have found that sensory and motor fields are specialized for different types of movement, such that in mice the motor cortex controlled the forward movement (protraction) of their whiskers and the sensory cortex controlled backwards movements (retraction) of whiskers. So if a whisker hits an object, then a reasonable first reaction might be a motor command for retraction. Similarly, the motor cortex stimulates protraction for more active exploration. Hence, the sensory cortex is also motor and the motor cortex is also sensory. In an ecological context, these combined reactions offer a repertoire useful for a mouse seeking food and shelter in a complex environment.

Promoting Apoptosis

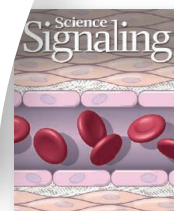
During acute disease, the promyelocytic leukemia (PML) protein becomes fused to another protein as a result of a chromosomal translocation. This protein appears to have multiple and varied functions, including the ability to form distinctive complexes in the nucleus that suppress tumorigenesis and promote apoptotic cell death. **Giorgi *et al.*** (p. 1247, published online 28 October; see the Perspective by **Culjkovic-Kraljacic and Borden**) have proposed a mechanism by which PML influences the cellular signals that promote apoptosis. The protein was localized at sites of contact between the endoplasmic reticulum and mitochondria, where it associated with a calcium channel, a protein kinase, and a protein phosphatase, to regulate calcium mobilization into the mitochondrion, which then triggers the cell death program.

Cellular Devices

Cellular control mechanisms might offer opportunities to build genetic devices capable of sensing aberrant cells and activate a regulatory signal that directs the cell to alter its biological state.

Culler *et al.* (p. 1251; see the Perspective by **Liu and Arkin**) present a proof of principle for a synthetic gene network in which cells were engineered to make an RNA-based device that detected molecules associated with disease states such as inflammation and cancer. Detection then triggered expression of a gene that made the cells more sensitive to a drug causing cell death.

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Alan I. Leshner is the chief executive officer of the American Association for the Advancement of Science and executive publisher of *Science*.

Protect U.S. Science Funding

THE RECENT POWER SHIFT IN THE U.S. CONGRESS REFLECTS IN PART THE PUBLIC'S DESIRE to get the U.S. economy quickly back on track and the federal budget under better control. In recognition, both the Obama Administration and the Republican Party leadership are considering significant budget reductions. These could result in 5 to 10% (or greater) cuts in R&D allocations for fiscal years 2011 and 2012. The consequences would be severe. Federal agencies, which often commit their funds years in advance but only pay them out in later years, would have little left for new and competing renewal grants. Agencies could see funding success rates fall to below 1 in 10 applications, and new investigators—the seeds of the future—could be hit even harder. These kinds of budget cuts work against the ultimate national goals of restoring the U.S. economy and its international prowess. It is well documented that science, engineering, and technology fuel innovation and economic growth. That is why virtually all competitor countries, including India, China, and Korea, are increasing investments in science and engineering research, development, and education. U.S. funding looks like it could be heading in the opposite direction.

The science and engineering community must mobilize now to stave off these funding cuts, which could be decided very soon. Scientists and engineers need to reach out and engage with the public and policy-makers about science's role in solving societal problems, particularly in fostering employment and economic growth. Policy-makers do respond to public perceptions and priorities. A similar educational approach helped to ensure the inclusion of research, development, and education in the American Recovery and Reinvestment Act (known as the Stimulus Bill) and in the America COMPETES Act. Both of these resulted in significant increases over the past few years in science and engineering research and education funding, but both are due to expire this year. More recently, the same kind of vigorous engagement helped to stave off potentially draconian science budget cuts in the United Kingdom, although not completely.

What would be most effective for the science and engineering community to do right now? They should reach out and speak to the public and policy-makers at both ends of the spectrum, from their local communities, local government leaders, and congressional representatives, to local and national media outlets and leaders of the Executive Branch. Policy-makers should be invited to visit laboratories and meet with scientists and engineers at all career stages and get a sense of their research programs and future goals. Research institutes should hold forums to educate the public on how science, engineering, and technology development are critical to solving almost every societal problem. Local industry leaders could help convey the message that research is critically important to innovation and economic growth. The case was laid out extremely well in two reports by the U.S. National Academies: *Rising Above the Gathering Storm* and its recent update, *Rapidly Approaching Category 5*. They provide a series of critical recommendations for exploiting the full power of science, engineering, and technology now and for the future, and they include a wealth of clearly stated information that can be used as talking points.*

Many scientists and engineers are reluctant to speak about their work to their neighbors and their policy-makers, fearing that they won't be understood or that it won't have an impact. But this is not a time for reticence, complacency, or helplessness. If scientists and engineers want public support and if they want to contribute fully to the betterment of humankind, they need to reach out to the public right now as a united community. It is critical that no single discipline or research agenda attempt to promote itself at the expense of another. Society can't achieve a better future if scientists, engineers, and the public sit back and allow short-sighted decisions to starve the engines of future problem-solving and innovation.

— Alan I. Leshner

10.1126/science.1200554



*www.nap.edu/catalog.php?record_id=12999



ECOLOGY

Frequency Matters

Aggressive conflict is dangerous. Thus many animals have evolved signals to advertise their ability to defend resources, such as antlers in elk or bird song. For many aquatic species, however, signaling one's prowess is not so straightforward. Murky environments hinder the ability of receivers to discern visual cues, making proactive signaling more difficult. Amazonian knife fish use an electric sense to navigate and forage in muddy tropical waters. Individuals produce distinct electric signals, which have been implicated in courtship and aggressive displays. Through a series of experiments conducted within a natural population of Amazonian knife fish (*Sternarchorhynchus* sp.) in Peru, Fugère *et al.* demonstrate that larger males produce higher-frequency signals and that fish that emit higher-frequency signals outcompete those with lower frequencies in direct competition. Furthermore, fish only respond aggressively towards an artificial electric signal played at a frequency lower than their own. Thus, the frequency of an animal's electric discharge accurately signals its resource-holding potential, and competitors heed the electrical warning. — SNV

Biol. Lett. 10.1098/rsbl.2010.0804 (2010).

SOCIOLOGY

Political Leapfrogging

Although there have been many discussions of the polarized nature of American politics, do the views of elected officials match the preferences of their electorate? Bafumi and Herron sought to answer this question by comparing a national opinion survey of American voters (the Cooperative Congressional Election Study; CCES) with legislator voting records of the 109th (2005–2006) and 110th (2007–2008) Congresses. In many cases, the CCES questions were similar to (or the same as) actual congressional roll call votes, which allowed for better comparison. By developing a linear scale bounded by representatives (or CCES respondents) who had taken consistently liberal or conservative positions, the authors found that members of Congress were more extreme than the voters they represented. The median member of the 109th House of Representatives was more conservative than the median American voter, but the median



member of the 110th House of Representatives was more liberal. Thus, voting out one extremist usually led to replacement by someone equally extreme, but of the opposite party. The authors refer to this as “leapfrogging” because the moderate views of the median American voter are leapfrogged during the turnover. Although the turnover was similar in the Senate, overall it appeared to be more moderate. — BJ

Amer. Polit. Sci. Rev. 104, 519 (2010).

BIOTECHNOLOGY

Expanding Archaeal Diet

Archaeal methanogens transform carbon dioxide into methane under an extraordinary range of conditions, unfazed by surrounding acids, bases, salts, or extremes of temperature. However, they are for the most part quite choosy about their substrate. Lessner *et al.* now show that by introducing an esterase enzyme (MekB) from an aerobic bacterium into the metabolic pathway of one such methanogen, they can extend the organism's diet to encompass methyl esters of acetate and propionate. On incorporation of a plasmid encoding MekB, *Methanosarcena acetivorans* proved capable of growing and emitting methane with either ester

acting as the sole carbon source. Methanol appeared to be the portion of the ester most readily reduced; acetate was transformed more slowly, whereas propionate simply accumulated as a by-product. The success of merging pathways from bacterial and archaeal domains, coupled with the effectiveness of the resulting engineered organisms in consuming common esters, bodes well for broader applications in processing biomass and organic waste products to form energy-dense natural gas. — JSY

mBio 1, e-00243-10 (2010).

BIOMEDICINE

NKT Cells Fight Cancer

One way tumors evade the immune system is by fostering an immunosuppressive environment. Although immune cells such as macrophages are known to contribute to this immunosuppression, whether neutrophils, an immune cell typically associated with inflammation, do too has not been fully explored. De Santo *et al.* now find that melanoma patients have elevated frequencies of immunosuppressive neutrophils in their blood. This correlated with increased concentrations of the acute-phase response protein, serum amyloid A 1 (SAA-1), in both the plasma and in tumors. In vitro, SAA-1 induced neutrophils from healthy donors to become immunosup-

pressive. As a way to modulate SAA-1–induced neutrophil differentiation, the authors exposed immunosuppressive neutrophils to natural killer T (NKT) cells, immune cells that can counteract immunosuppression. SAA-1 promoted the interaction of NKT cells with neutrophils, which resulted in neutrophils adopting a proinflammatory phenotype. These results suggest that adoptive NKT cell therapy may be one way to relieve tumor-induced immunosuppression. — KLM

Nat. Immunol. **11**, 1039 (2010).

MATERIALS SCIENCE

1...2...3...I Love to Count

Scanning transmission electron microscopy (STEM) methods are highly sensitive to atom type and arrangement and thus can be used for precise analysis of the structure of materials at the nanometer scale. However, current applications have been limited by the need for calibration standards that exhibit complex relationships between the image intensity and sample thickness, orientation, and crystallinity, or depend on comparisons of relative contrast. LeBeau *et al.* have developed a method to perform a column-by-column count of the atoms in an arbitrarily shaped sample without prior knowledge of the shape or thickness, by making direct comparisons with simulated images. Position-averaged convergent beam electron diffraction patterns were used to measure the local thickness and ensure accurate tilting of a gold foil sample. The raw data were filtered to obtain the location of each atom column, and comparisons between experimental and simulated column intensities were used to determine the number of atoms within each column to within ± 1 atom. By varying the value used in the simulations for the finite effective source of the microscope, a parameter that is difficult to measure in non-aberration-corrected STEM, it was also possible to determine when the simulated and observed intensities did not agree, and thus to obtain limits for this parameter. In conjunction with other atomic-level microscopy techniques, it should be possible to count the atoms in more complex samples without the need for calibration standards. — MSL

Nano Lett. **10**, 4405 (2010).

on this motif in which they expanded the layer spacing of a potassium lithium titanate sample by inserting alkane thiols and then reductively assembling gold nanoparticles between them. They deduced by electron microscopy that the solid contained intercalated gold disks less than 1 nm thick and about 3.5 nm wide. They then monitored the capacity of the assembled material to selectively oxidize benzene to phenol in water upon visible irradiation (at wavelengths exceeding 420 nm) at room temperature. Although the catalysts adsorbed benzene, they initially showed little selective activity. However, when phenol was added at the outset, the product enhanced its own formation pathway: Photoactivity increased substantially to a net phenol yield of 62% at a selectivity of 96%. — PDS

J. Am. Chem. Soc. **132**, 10.1021/ja1083514 (2010).

BOTANY

One in the Same, Almost

Although many flowering plants reproduce by sexual reproduction, some plants evade the complexities of chromosome reduction, dispersion of gametes, and fertilization by undergoing a type of asexual reproduction called apomixis. Such plants produce a seed, but that seed carries the same diploid genome as its one parent and can generate a new plant. Apomixis has



been suggested to be a deregulated form of sexual reproduction. Garcia-Aguilar *et al.* have now analyzed the molecular mechanisms that distinguish the sexual reproduction pathway found in maize from the apomictic pathway found

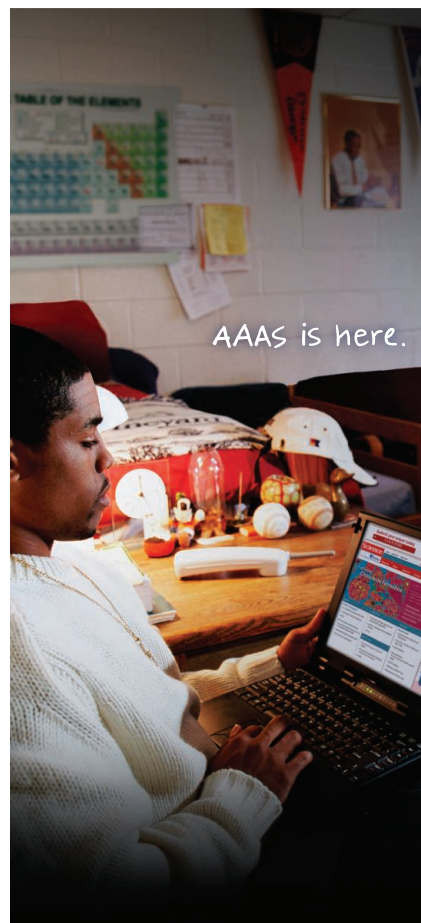
in a wild relative of maize. The results implicate an alteration in DNA methylation pathways, which normally impose repressive marks on the chromatin. When certain methyltransferases expressed during sexual reproduction function poorly, or are deleted, the chromatin takes on a state that is more permissive of transcription, and a developmental program resembling apomixis is seen. Thus, chromatin modifications and their subsequent effects on gene transcription are involved in determining whether a plant reproduces sexually or by apomixis. — PJH

Plant Cell 10.1105/tpc.109.072181 (2010).

CHEMISTRY

Visible Product Assistance

Adsorption of gold nanoparticles to titania surfaces has proven a promising approach to extending the photocatalytic properties of a semiconductor from the ultraviolet into the visible regime. Ide *et al.* explored a variation



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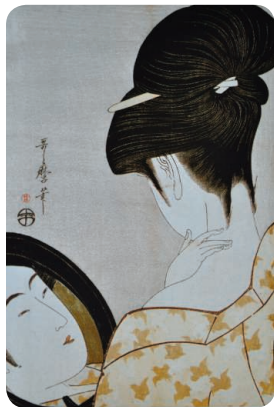
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Deathly Pale

Did makeup change the course of Japanese history?

Possibly, say researchers studying bones from a cemetery reserved for samurai, the nobility during the 1603-to-1867 reign of the Tokugawa military dynasty. Using x-rays and atomic absorption spectrometry, the group found that the bones of infants contained 50 times as much lead by weight as the bones of women did; women's bones, in turn, had higher lead concentrations than those of men. The infants probably suffered lead poisoning, which can cause organ failure and neurological problems, especially in children.

In Tokugawa-era Japan, upper-class women whitened their faces with a lead-based cosmetic. Children of the nobility could have ingested the makeup while nursing, Tamiji Nakashima, an anatomist at the University of Occupational and



Environmental Health in Kitakyushu, Japan, and colleagues write in the January 2011 issue of the *Journal of Archaeological Science*. They add that early exposure to lead "may have left [adult samurai] intellectually incapable of dealing with the political crisis" that led to the Tokugawas' demise.

Other research also fingers makeup as a source of lead exposure, says Koji Naruse, an archaeologist at the University of Tokyo not involved in the study. But he says it is unclear how infants would have acquired so much more lead in their bones than their mothers had. And the idea that lead contributed to political upheaval is "a fascinating stretch," says Jeffrey Kingston, a historian at Temple University's Japan campus in Tokyo. He notes that the rival samurai who overthrew the Tokugawa clan probably had the same childhood exposure to lead.

So Nice, They Dug Him Up Twice



Last week in Prague, inside the chilly Church of Our Lady Before Týn, cameras flashed as workers lifted a coffin from its crypt. Inside lay Tycho Brahe, the medieval astronomer whose copious observations helped Johannes Kepler formulate his planetary laws and whose 1601 death still has historians puzzled.

Legend has it that Brahe died of a burst bladder because he was too polite to leave a banquet to urinate, but analysis of beard hairs from a 1901 exhumation found mercury, suggesting he died of poisoning.

In 2001, medieval archaeologist Jens Vellev (right) of Aarhus University in Denmark asked a priest at the church about reopening the vault to find out for sure. Getting permission from city authorities, however, turned into a 9-year-long slog that Vellev



compares, without bitterness, to a Kafka novel. "It was an interesting experience to have the possibility to talk to so many strange and interesting people in so many strange offices," he says.

Now Vellev and his colleagues will subject the remains to tests including CT scans and PIXE analysis, which show the elemental makeup of a sample. Vellev doesn't suspect foul play. Instead, he wants to find out what medications the famously eccentric astronomer was taking when he died. Brahe liked to mix his own, Vellev says, and might have done himself in.

THEY SAID IT

"To a deity, the big bang is very sexy. ... And with the LHC, we can now simulate it at least as accurately as a porn star can fake an orgasm."

—Artist Jonathon Keats explains his latest work, *Pornography for God*, a special altar in a Brooklyn art gallery displaying a live feed of particle collisions from the Large Hadron Collider.

SURFACING

These grinning faces, carved into rock by prehistoric people, emerged from the waters of Brazil's Rio Negro last month when a drought brought the Amazon tributary to its lowest level since 1902.

In the Amazon, rock etchings turn up most often near waterfalls or rapids and stay submerged during the rainy season; some appear only once in a century. The petroglyphs discovered in October were visible for only a week, says Akira Tanaka, a manager at SGI Brazil, which runs an environmental preserve on the land.

Archaeologists didn't rush to the scene, but if they had, they probably would have found few clues, says archaeologist Edithe Pereira of the Museu Paraense Emilio Goeldi in Belém. "Once submerged, any nearby evidence is long gone. There is nothing to excavate," says Pereira, who, as one of only a few petroglyph researchers in Brazil, has organized diving expeditions to inspect submerged carvings. Partly as a result, she says, only two Amazon carving sites have ever been dated, the older to 11,000 years of age. And a dozen large dams being constructed in the region mean "a lot of sites are going to go underwater forever."

Carvings in the American Southwest and elsewhere often show animals, but Amazonian artists preferred smiling or frowning faces. Perhaps, Pereira speculates, as ancient man settled down, "he becomes more important than the animals, so he represents himself, or a shaman."



NEWS ANALYSIS

New Republicans Could Revise Party Line on Research Funding

Since the midterm elections, U.S. science leaders have been reassuring their community that Republicans traditionally have been very supportive of basic research. As proof, they point to statements from Newt Gingrich, former speaker of the House of Representatives, that his biggest failure after the 1994 Republican takeover of Congress was not pushing through a tripling of the National Science Foundation (NSF) budget.

But the new House leadership, due in part to the success of Tea Party activists, is quite different from its predecessors. And that difference could put a damper on hopes that the next Congress will deliver on a long-promised expansion of federal spending on research and education outlined in a 2005 National Academies' report that has been embraced by both parties. Here's why.

The day after Republicans reclaimed the House of Representatives, their second-in-command, Representative Eric Cantor (R-VA), spelled out what's changed. "Perhaps the single greatest criticism of our previous majority," Cantor explained in a 22-page governance plan, "is that *we spent too much* and that *we grew the size of government*." (His emphasis.) Those mistakes were a result of ignoring "our core Republican principle of limited government," Cantor noted before adding, "We're not the same Republican Party."

Of course, limited government and reduced spending are not what Norman Augustine, former CEO of Lockheed Martin, and the other authors had in mind in their influential 2005 report, *Rising Above the Gathering Storm*. Instead, they called for a \$19-billion-a-year increase by 2013 in the federal investment in research, education, and innovation to keep the United States competitive in a

global economy. "Actions such as doubling the research budget are investments that will need to be made if the nation is to maintain the economic strength to provide for its citizens' health care, social security, national security, and more," declares a recent, 5-year update of the original report that deplores the lack of progress toward its goals.

The 2005 report offered four major recommendations. The request for more research spending received the most attention—in particular, a 10%-a-year increase in the budgets for NSF, the Department of Energy Office of



"What I hear from new members is that 'we love science, but we are coming to Washington to cut the budget.' Well, to that I say, 'When you're designing a plane and trying to save weight, you don't throw out the engine.'"

—NORMAN AUGUSTINE, CHAIR,
RISING ABOVE THE GATHERING STORM PANEL

Science, and the National Institute of Standards and Technology and the creation of the Advanced Research Projects Agency–Energy within DOE. But an even higher priority for the panel was expanding the federal role in training new science and math teachers and improving the skills of those already in the classroom. [The President's Council of Advisors on Science and Technology (PCAST) recently fleshed out some of those approaches to improving elementary and secondary education (*Science*, 24 September, p. 1582)]. The report also recommended ways to attract more U.S. students into science and engineering, and changes in immigration and patent policies so that U.S. companies can compete more effectively in world markets. In other words, it said, a healthy U.S. innovation climate requires more spending and a larger role for government.

The report was requested by a bipartisan

coalition of legislators in a Congress controlled by Republicans, and its words provided the basis for the 2007 America COMPETES Act. Science lobbyists invoke both the 2005 academies' report and the 2007 legislation, which is up for reauthorization this year, as evidence of bipartisan support for basic research. And they have extrapolated that attitude to the 112th Congress that will convene in January with a sizable Republican majority in the House and a reduced Democratic presence in the Senate. Some science advocates also speculate about a left-right consensus that would yield big increases for energy research.

But all that may be wishful thinking. "The nation's outlook has worsened. ... Our public school system has shown little sign of improvement, particularly in mathematics and science," complains the updated *Gathering Storm* report, subtitled *Rapidly Approaching Category 5*. There are more, not fewer, constraints on the nation's capacity to innovate, it notes, resulting in a decreased ability to provide good jobs. Although the report doesn't say it, that deterioration occurred despite 4 years of a Democratic Congress whose most visible leader, House Speaker Nancy Pelosi, had a simple prescription for healing the country's ailing economy: science, science, science, and science.

Nobody's expecting any such focus on science from the new speaker, Representative John Boehner (R-OH), or any of his leadership team. Neither Cantor's statement nor the more visible *Pledge to America* that the party issued during the fall campaign even mentions science, research, or education. The only reference to "innovation" comes as part of a passage in the *Pledge* on how "excessive federal regulation ... hampers innovation and postpones investment in the economy." In other words, get the government off our backs so that the private sector can do its thing.

As a former captain of industry, Augustine is comfortable in the company of those making such arguments. "I'm a registered Republican, although I try to remain nonpartisan," he says. "I've spoken with most of the key players. And I agree that there are lots of places where the federal budget can be cut."

What worries Augustine is the idea that everything should be on the chopping block.

“What I hear from new members is that ‘we love science, but we are coming to Washington to cut the budget.’ Well, to that I say, ‘When you’re designing a plane and trying to save weight, you don’t throw out the engine.’ The biggest threat to innovation is this army of pickaxes that want to sweep away everything, including research.”

Such broad cuts would devastate the U.S. science enterprise, says Chuck Vest, the president of the National Academy of Engineering and a member of both the 2005 and 2010 panels. Briefing PCAST on the updated report a few days after the election, Vest warned that tough times are ahead and cited the report’s opening line from physics Nobel laureate Ernest Rutherford: “Gentlemen, we have run out of money. It is time to start thinking.” If the panel’s recommendations remain unheeded, he added, another update in 2015 could begin with an even darker forecast, which he attributed hypothetically to John Holdren, science adviser to President Barack Obama: “Gentlemen, we have run out

“The single greatest criticism of our previous majority is that we spent too much and that we grew the size of government. We’re not the same Republican Party.”

**—ERIC CANTOR,
THE NEXT HOUSE MAJORITY LEADER**



of money. It is time to start drinking.”

What the Republicans are thinking may not be visible during the lame-duck Congress. The chief item of business is a spending bill for the remainder of the 2011 fiscal year, which began on 1 October. If Democrats try to wrap all 12 appropriations measures into one omnibus package, several science agencies could receive some portion of the increased spending that Obama requested in his February budget submission to Congress. More likely, however, is a further extension of 2010 levels.

Reauthorization of the 2007 COMPETES bill before Congress adjourns would strengthen the hand of the Obama Administration in any upcoming battles with Republicans over science spending. But prospects for the bill, which passed the House in May, seem dim. “The vote [in 2007] was not at all partisan,” notes Representative Rush Holt (D–NJ), a former physicist and strong advocate for the bill. “But I’m afraid

that some Republicans see it as a Democratic initiative that grew out of the speaker’s innovation agenda. That would be enough of a reason to oppose it.”

Whatever happens in the next few weeks, the Republican campaign for a smaller government is expected to kick off in earnest in January. And if advocates for continued federal investments in research and education are blown out of the water, the culprit may be a Category 5 electoral hurricane.

—JEFFREY MERVIS

DRUG RESEARCH

Roche Exits RNAi Field, Cuts 4800 Jobs

The Swiss drug company Roche announced last week that it is stepping away from research in RNA interference (RNAi), a popular approach to medical therapies and one that Roche has poured more than \$400 million into over 3 years. The decision is part of a plan to reduce Roche’s workforce by 6%, or 4800 people.

Although it’s not unusual to see contractions in big pharma, Roche’s decision to abandon work in RNAi is striking because the field has gotten much attention. The technique, which earned a Nobel Prize in 2006 (and *Science*’s Breakthrough of the Year in 2002), involves using tiny RNA molecules to shut down specific genes. But getting them to diseased tissues in the proper dose has challenged scientists. The field is “fascinating, but unfortunately this delivery point is a hurdle,” says Claudia Schmitt, a Roche spokesperson. The 50 or so employees at Roche’s RNAi “Center of Excellence” in Kulmbach, Germany, most of them scientists, were just

not able to overcome that stumbling block, she adds.

No RNAi drugs are on the market yet; one for macular degeneration failed in late-stage trials last year. But more than a dozen clinical trials are under way in cancer, asthma, and other conditions. “People are going to look at this Roche thing and ... be less enthusiastic” about RNAi, says Mark Kay, a gene therapist at Stanford University in Palo Alto, California, who’s been working with the technique in hepatitis C. That would be a shame, Kay argues. A veteran of bitter setbacks in gene therapy, he wasn’t expecting RNAi to advance nearly as rapidly as it has, and he still considers it promising.

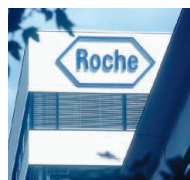
Delivering RNAs to target tissue remains tough, although researchers are making progress. Chemically modifying one strand of the double-stranded small RNAs has reduced off-target effects, such as the molecules’ unnerving propensity to hit too many gene sequences

(*Science*, 12 November 2004, p. 1124). This may also ease another glitch: Depending on how they’re packaged, the RNAs and their delivery vehicle together can stimulate the immune system in dangerous ways. “You are going to run into technical barriers,” says Kay. “It takes time to solve them.”

Like many other pharmaceutical companies, Roche was in a tight spot, with a bet that wasn’t about to pay off. Even if RNAi pans out, it isn’t likely to offer a windfall in the next year or two. “The easiest things to let go are things that have long-term perspectives,” says John Maraganore, the chief executive officer of Alnylam, an RNAi biotech company in Cambridge, Massachusetts. Roche formed a collaboration in 2007 with Alnylam, which once owned the German site now impacted by cuts. Alnylam currently has three RNAi drugs in clinical trials.

Other large drug companies remain invested in RNAi, for now at least, including Merck, Novartis, and Takeda.

—JENNIFER COUZIN-FRANKEL



ScienceInsider

From the *Science*
Policy Blog

The British government has apologized for postmortem **research done on nuclear plant workers** without proper consent in a report that blames British pathologists for ethical lapses in scores of research projects carried out from the 1950s to the early 1990s. An official inquiry launched in 2007 notes that the work generated important data. <http://scim.ag/redfern-apology>

A series of ill-advised decisions by operators to save time and money likely contributed to the eventual **blowout and explosion that oiled the Gulf of Mexico** this year, says an expert panel that found nothing inherent in deepwater drilling or the geology of the offshore oil and gas reservoirs that would have doomed the *Deepwater Horizon*. One example, it says, was relying on a newly installed cement plug in the well despite the plug having failed three successive tests. <http://scim.ag/poor-decisions>

A budget impasse is jeopardizing a hard-won compromise on how to handle Europe's share of additional costs for **the international ITER fusion reactor**. ITER chief Osamu Motojima discussed with *Science* this week his plans to create a larger optional reduction without creating technical risks. The details must be worked out in the next few months to keep ITER on track. http://scim.ag/Motojima_ITER

ScienceInsider provided a continuously updated, blow-by-blow account of a **climate change hearing**, attracting hundreds of viewers and comments. Billed as a "rational discussion" of the issue by the House of Representatives science committee, it was a fare-well performance for Democrats before the Republicans assume control of the panel in January. <http://scim.ag/live-climate-hearing>

Croatian scientists are up in arms over a set of new policies instituted by the science ministry last month. The policies include merging the research and higher education "councils" and eliminating an ethics body altogether. The ministry says it is reviewing more than 1000 comments. http://scim.ag/croatian_cuts

For more science policy news, visit <http://news.sciencemag.org/scienceinsider>.

PALEONTOLOGY

Excavation Yields Tantalizing Hints
Of Earliest Marine Reptiles

CHAOHU, CHINA—Eons ago, convulsions warped the land here on the edge of town, thrusting slabs of mudstone and limestone high into the sky. Buried in the rock are traces of a mass extinction that wiped out about 90% of oceanic life forms and brought the Paleozoic era to a shuddering close 251 million years ago—the end of the Permian period and beginning of the Triassic. When life rebounded in the early Triassic, a new kind of top predator arose: ichthyosaurs. Marine reptiles would dominate the oceans until another mass extinction ended the Cretaceous 65 million years ago.

The origins of marine reptiles are an enigma. Their ancestors came from the land, but scientists can only imagine what sort of animals ventured into the sea and evolved into the three successful lineages of marine reptiles: sleek tuna-shaped ichthyosaurs; sauropterygians, including the long-necked plesiosaurs; and mosasaurs, which gulped down prey with double-hinged jaws like those of snakes. "The invasion must have happened very fast, within 4 million or 5 million years after the end of the Permian," says paleontologist Jiang Da-yong of Peking University in Beijing. But the fossil record

of this ocean colonization is largely a blank slate. "We have very little material from the lower Triassic," says Olivier Rieppel, a specialist on Mesozoic marine reptiles at The Field Museum in Chicago, Illinois.

Answers may be entombed at Majiashan quarry, in a 150-meter-thick outcropping dappled with shades of gray that spans 6 million years of geologic history. "We expect to find new species here," including forms marking the land-sea transition, says Ryosuke Motani, a paleontologist at the University of California, Davis, who, with Jiang in September, launched the first systematic excavation at Majiashan. The dig's first fruits—including an ancestor of the plesiosaurs—are already generating a buzz.

Majiashan, north of Chaohu City in central China's Anhui Province, is a fixture on the paleontological map. In 1972, railway workers here found an unusual fossil that two scientists at the Institute of Vertebrate Paleontology and Paleoanthropology in Beijing—Young Chung-chien and Dong Zhi-ming—identified as a new species of ichthyosaur, *Chaohusaurus geishanensis*. Since then, a couple dozen more *Chaohusaurus* skeletons from the early Triassic

have streamed out of this site and nearby Guishan; most were unearthed by quarry workers and farmers, and most have ended up in private collections. Besides looking more like land reptiles than other ichthyosaurs, *Chaohusaurus* was about 70 centimeters long—much smaller than ichthyosaurs from the late Triassic and the Jurassic, which could top 15 meters in length.

Motani and Jiang have their eyes on a bigger prize: undiscovered pint-sized marine reptiles, presumed to be tens of centimeters long, with vestigial limbs, or other terrestrial features such as lungs and ears better adapted to life on shore. "We hope to find the oldest marine reptiles. Then we'll look for signs of their ancestors," says Jiang. Worldwide, few outcroppings from the Permian-Triassic boundary, the beginning of the Mesozoic era, are as promising



Rescue paleontology. Ryosuke Motani (left) and Jiang Da-yong hope to unearth spectacular marine reptiles before farmers do.

ScienceNOW

as Chaohu. “This is where the fossils have to be,” says Nick Fraser, a paleontologist at National Museums Scotland in Edinburgh. “But you still have to get lucky to find them.”

The first fossil fragments of a marine reptile—those of an ichthyosaur—were unearthed at the turn of the 18th century. The species drew scientific interest only a century later, in 1811, when a 12-year-old girl collected the skull and vertebrae of *Temnodontosaurus platyodon* on the Dorset coast in England. Mary Anning became one of the great fossil hunters of all time. Among her many other discoveries is the first fossil of a plesiosaur, *Plesiosaurus dolichodeirus*, which she found in 1821. The creature was so unlike living animals that it helped convince skeptics that species could go extinct—a new and not widely accepted concept at the time.

Since then, several dozen species of extinct marine reptiles have come to light, mostly members of the three dominant lineages. Ichthyosaurs, soon after arising in the early Triassic, morphed into a variety of body plans. It took about 20 million years, however, for plesiosaurs—four-flippered behemoths resembling overgrown sea lions—to have evolved from early sauropterygians. The ancestors of mosasaurs, which arose in the late Cretaceous, are entirely obscure, as is the kinship of a less-known fourth lineage of marine reptiles, the lizard-like thalattosaurs of the middle to late Triassic, which appear in the fossil record from 247 million to about 200 million years ago.

Researchers are beginning to get a handle on the physiology of these beasts. Recent findings suggest that ichthyosaurs and plesiosaurs—which cruised for food—were warm-blooded, allowing them to maintain constant muscle activity (*Science*, 11 June, p. 1379; p. 1361 depicts evolutionary relationships between Mesozoic marine reptiles). Mosasaurs were ambush predators more like crocodiles, their body temperature matching that of the water.

Plenty of questions about early Mesozoic marine species remain unanswered, and Anhui holds particular appeal as a possible source of information. During the Triassic, two large oceans intersected in this region: the Panthalassa, which covered half the globe, and the Tethys, which gave rise to the Indian Ocean and the Mediterranean Sea. Bivalves from both oceans have been found in Early Triassic sediments in Chaohu, Motani says.

On an exploratory trip to Majiashan in September 2008, Jiang tracked down farmers and quarry workers who had collected



Fine pedigree. Majiashan is famed for *Chaohusaurus*, an early diminutive ichthyosaur.

fossils from the site and was shown some intriguing specimens. “The farmers called them all *Chaohusaurus*, but it’s clear that some are other kinds of reptiles,” Jiang says. The skeletal fragments were too big to be *Chaohusaurus*; in Jiang’s estimation, they represent at least two new species.

The researchers are racing to recover more of these treasures, but time is not on their side. Over the years, quarry workers at Majiashan have gouged deeply into the Permian-Triassic boundary formation. “We only have this much limestone,” says Motani, gesturing to an outcropping with spray-painted red splotches indicating layers that have been dated. “When it’s gone, the early Triassic is gone.” But his team has a big advantage: The geological layers here are stacked like books on a shelf, which makes it possible to survey millions of years of rock without climbing high. And specimens can easily be carted out after excavation.

To protect the site, the Chaohu government has vowed to close the quarry and the nearby cement factory it supplies, says Jiang. And to cut down on illegal fossil sales, his team, working with Jiang Li-ai, director of Anhui Geological Museum in Hefei and president of the Paleontological Society of Anhui, plans to educate residents and local authorities about the fossils’ scientific value.

Back in Beijing, Jiang Da-yong has begun analyzing the specimens pried from the rock in September. They include the remains of a new kind of early ichthyosaur and skeletal fragments of a sauropterygian that was less than a meter long—a tiny ancestor of the mighty plesiosaurs. “It’s exciting,” says Rieppel. “We are all hoping for more!”

—RICHARD STONE

From *Science’s* Online Daily News Site

Evolutionary Relationships Hold, Even in Our Guts

The human body is coated with bacterial cells. They live on our skin and between our teeth. They particularly like our warm, nutrient-filled gut, where they help digest food, make vitamins, and produce some seriously smelly gas. But when it comes to these gut bacteria, we are not what we eat. A new analysis of feces from humans and several other primates, published in *PLoS Biology*, finds that evolutionary history, not diet, determines the makeup of our intestinal bugs. <http://scim.ag/gut-bugs>

Spacecraft Successfully Returns Asteroid Dust

For the first time, a spacecraft has landed on and returned samples from a celestial body other than the moon. Last week, ecstatic Japanese officials confirmed that dust retrieved from the capsule of its Hayabusa spacecraft did indeed come from the asteroid Itokawa and is not earthly contamination. The microscopic bits had been under investigation since the capsule landed in the Australian outback last June after a trouble-plagued, 7-year roundtrip to the lumpy, potato-shaped asteroid. <http://scim.ag/asteroid-dust>



Fish Sleep Soundly in Mucous Cocoons

Even the ocean has bedbugs. Tiny blood-sucking crustaceans roam the seas, nipping at the scales of passing fish. But the parrotfish (*Chlorurus sordidus*) has evolved an unusual defense. Researchers report in the *Proceedings of the Royal Society B* that the fish spend up to an hour—and 2 % of their daily energy budget—spinning cocoons from their own mucous before they settle down to slumber for the night. These transparent, gelatinous balls of spit are large enough to envelope the fish from head to tail. Researchers showed that fish sleeping without protection were 80% more likely to be bitten by the crustaceans. <http://scim.ag/cocoons>

Read the full postings, comments, and more at <http://news.sciencemag.org/sciencenow>.

BIOETHICS

Oversight But No Strict Rules for Synthetic Biology

A presidential bioethics commission concluded this week that the U.S. government should not clamp down too hard on research on synthetic biology, a young field that it says doesn't yet pose serious risks. Instead, the commission will recommend better coordination of research and ongoing reviews to see if rules should be updated.

Formed in April, the 13-member Commission for the Study of Bioethical Issues' first assignment was prompted by a report in *Science*: the insertion of a synthetic genome into a bacterium by a team led by biologist J. Craig Venter (*Science*, 21 May, p. 958). In July and September, the panel heard from various experts about the concerns and potential benefits of that development and other types of synthetic biology (*Science*, 16 July, p. 264).

The commission met last week in Atlanta to wrap up a report due on 15 December. Members emphasized the need to strike a balance between what they called "letting science rip" and the precautionary principle of waiting until all risks are understood, explains co-chair Amy Gutmann, president of the University of Pennsylvania. Although the chances are low that an organism made with synthetic biology will be accidentally released, such an accident could have a big impact, the panel concluded. So the draft report calls for "prudent vigilance": encour-



Open door. Gutmann wants dialogue about risks.

age research while monitoring the field and updating regulations as needed.

Such oversight could be done by a coordinating committee that reports to the White House Office of Science and Technology Policy. It would evaluate government funding and conduct a "gap analysis" of existing regulations, as well as review the need to revise patent and data-sharing policies.

The panel's 19 draft recommendations

include some specific advice. New synthetic organisms should be marked so they can be tracked if they escape into the wild and should be made wimpy so that they couldn't spread. Biosafety training should extend to engineers and others doing synthetic biology. The commission is also concerned about public perceptions—many media reports inaccurately suggested Venter had "created life," Gutmann notes. She says the report will suggest that a nonprofit group create a Web site for examining biotechnology claims along the lines of Factcheck.org, which corrects misstatements by politicians.

The commission agreed that the government should keep an eye on an estimated 2000 amateur synthetic biologists, known as Do-It-Yourselfers (DIYers). Federal officials might eventually want to require that everyone comply with rules such as the National Institutes of Health's guidelines for studying recombinant DNA even if they don't receive NIH funding. But the panel decided against any immediate mandates that could drive the hobbyists underground. Instead of "us versus them," the commission wants the DIYers "to be invited to the table," says Gutmann.

"The direction they're taking seems to me to be the right one," says ethicist Thomas Murray of The Hastings Center in Garrison, New York.

—JOCELYN KAISER

NATIONAL INSTITUTES OF HEALTH

Collins Endorses Merger of U.S. Addiction Research Programs

The National Institutes of Health (NIH) will likely dissolve its two institutes that study drug and alcohol abuse and combine their programs. In a statement last week, Director Francis Collins said he had received a formal recommendation from an NIH advisory board to create a single "new institute" to study substance use, abuse, and addiction.

The proposal "makes scientific sense," Collins said. A task force will figure out by next summer how to carry out the effective merger of the National Institute on Drug Abuse (NIDA) and the National Institute on Alcohol Abuse and Alcoholism (NIAAA).

Collins' decision is controversial. The \$462 million NIAAA budget is less than half that of the \$1.06 billion NIDA, yet it funds a slightly higher proportion of submitted grants. Alcoholism researchers and patient advocates are worried that their smaller institute will be swallowed up and

that certain studies, such as research on alcoholism-related liver disease, will be cut short. The NIAAA council voted unanimously earlier this year against a merger; NIDA's council members all voted in favor.

According to the advisory panel's report, the new institute would also house the National Cancer Institute's tobacco addiction programs. And nonaddiction research would go elsewhere—for example, NIAAA's research on fetal alcohol syndrome would likely be transferred to NIH's child health institute.

Critics of the plan say they're concerned that the problem of alcohol abuse will lose visibility with both the public and the research communities. Robert Messing of the University of California, San Francisco, says alcoholism research was "a backwater" before NIAAA was created but now attracts many young scientists. Former NIAAA Director

Enoch Gordis says "I believe it's not a good idea" because it ignores scientific differences between alcohol and drug addiction and the fact that alcohol kills far more people than drugs. "I just hope it doesn't do any damage."

The notion of combining the two institutes was suggested by a National Academies panel in 2003. But it was not until Congress created a Scientific Management Review Board (SMRB) in 2006, aimed at examining NIH's structure, that things began to happen. In September, an SMRB working group recommended either combining NIDA and NIAAA in a new institute or better coordinating addiction research across NIH. The full board voted 12–3 in favor of the new institute.

It's not a done deal, however. Secretary of Health and Human Services Kathleen Sebelius has to approve the merger plan and then notify Congress, which will have 180 days to intervene.

—JOCELYN KAISER

NEUROSCIENCE

New Clues About What Makes The Human Brain Special

What differentiates the human brain from those of our nearest primate relatives? Size, for one. Our brains are more than three times bigger than those of chimpanzees. But many researchers have come to suspect that less obvious differences lurking in the fine-scale structure of the human brain also contribute to its extraordinary processing power (*Science*, 2 March 2007, p. 1208). Identifying such microscopic differences has been a challenge, however.

Now, researchers who've examined preserved samples of cerebral cortex from humans and several species of ape say they've found some intriguing clues about what makes the human brain unique. They report that in a particular region of the prefrontal cortex, an area that contributes to abstract thinking and other sophisticated cognition, neurons have more space between them in the human brain than in the brains of apes. This extra space allows more room for connections between neurons, explains Katerina Semendeferi, a physical anthropologist at the University of California, San Diego, who led the new study along with graduate student Kate Teffer. More complex neural wiring presumably allows more complex information processing and, in turn, more complex cognition, Semendeferi says. The findings were presented last week at the annual meeting of the Society for Neuroscience in San Diego and published online this week in *Cerebral Cortex*.

"For me, it's very interesting," says Pasko Rakic, a neuroscientist at Yale University. Rakic notes that much of what distinguishes humans from other apes is the type of social behavior mediated by the prefrontal cortex. "This is the area where we have to look for differences."

Teffer, Semendeferi, and colleagues examined preserved brain tissue from all six living species of the hominid family, including eight humans, three chimpanzees, two bonobos, one gorilla, three orangutans, and two gibbons. The ape brains came from animals that died of natural causes at zoos or at a primate research center.

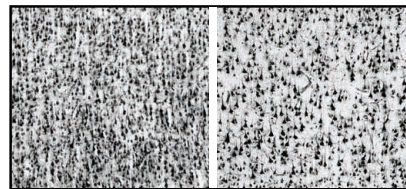
After slicing the brain tissue into ultrathin sections, the researchers selected four regions of the cortex for closer study. Three of these areas specialize in what might be considered more routine functions: the primary visual cortex (which makes sense of what we see), the primary somatosensory

cortex (which handles information about the sense of touch), and the primary motor cortex (which helps plan and execute movements). The fourth area, called Brodmann area 10 (BA10), sits at the pole of the frontal lobes, just above the eyes, and is thought to be involved in more complex types of thought. A debate has simmered over whether the frontal cortex as a whole is proportionally larger in humans than in other primates, but Semendeferi has reported previously that the BA10 subregion does seem to be expanded in humans.

The team used two methods to assess the spacing between neurons in the four areas of the cortex, based on images captured by a camera attached to a microscope. In one



Take a look. More space between neurons in the human brain (right) compared with the chimp brain (left) could allow more complex neural wiring.



approach, a computer program measured the horizontal distance between cell bodies, which appear as dark dots in the images (see above). Most neurons have a characteristic shape: a bloblike cell body with many protruding branches. These are the axons and dendrites that send and receive messages from other neurons. In the second approach, the computer calculated the proportion of the total area of an image that was occupied by cell bodies. As the spacing between cells increases, the horizontal distance increases and the area occupied by cell bodies decreases.

According to both measures, there were only subtle differences among the six species in the visual, somatosensory, and motor cor-

tices. The frontal lobes told a different story, however. In the human brain, cells in BA10 were more widely spaced than they were in any of the other apes.

"It's a well-done piece" of work, says Bob Jacobs, a neuroanatomist at Colorado College in Colorado Springs. "It's another in a long line of findings showing there's something special about the human frontal lobes."

Those findings include the observation that the dendrites of neurons in the human frontal cortex branch in more elaborate patterns than do those in other primates, as well as the observation that human frontal cortex, including BA10, contains a higher concentration of so-called Von Economo neurons, which some researchers believe are a type of high-performance neuron specialized for rapidly transmitting information from one brain region to another.

Because the new study focused specifically on the layer of the cortex that gives rise to connections between regions of cortex, the findings suggest that neurons in the human frontal cortex may also be more interconnected with their counterparts in other regions of cortex, says Chet Sherwood, an evolutionary neuroanatomist at George Washington University in Washington, D.C.: "They're going to be able to do computationally greater things because they're integrating more inputs." But Sherwood says the study leaves open the question of whether this wiring pattern arose as a direct result of natural selection or as a side effect of brain expansion in the human lineage.

Not everyone is impressed with the study, however, Manuel Casanova who studies brain evolution at the University of Louisville in Kentucky, sees a host of methodological problems, including the small number of samples, the way the tissue was prepared, and the algorithms used to measure the distances between cells. "It really seems to be a preliminary study whose conclusions may not generalize," he says.

Sherwood agrees that the study has some limitations, but he doesn't think they're serious enough to discard the findings. "I think there is a story there," he says. "There is a consistency with some of the other data, which gives it that whiff of repeatability."

—GREG MILLER

Bugs galore. We all harbor bacteria, but asthmatics host different species, including *Staphylococcus aureus*.

Bacteria and Asthma: Untangling the Links

Our guts and airways are awash in bacteria—but people with asthma have a different balance of microbes. Could this be a cause of disease?

SIX YEARS AGO, GARY HUFFNAGLE, AN immunologist at the University of Michigan, Ann Arbor, conducted an experiment that reflects what happens to many of us early in life. He exposed mice to a triple whammy: yeast in their intestines, mold spores up their noses that migrated down the airways, and an antibiotic drug. The animals began showing signs of asthma; blood tests revealed disruption of their immune systems.

“They developed some fairly wicked allergic disease in the lung,” says Huffnagle. The mold and yeast alone didn’t make much difference. “The bottom line is, those animals are perfectly healthy until we hit them with an antibiotic.”

Huffnagle’s study, published in 2004 in *Infection and Immunity*, was one of the first pieces in a dizzying new jigsaw puzzle of asthma, whose causes remain elusive even as the number of cases is soaring. Researchers have some striking clues: For example, children on farms are much less likely to get the lung disease. “A rich microbial environment in childhood is somehow protective,” says William Cookson, a respiratory physician and geneticist at Imperial College London. Cookson and others are now moving from the outside environment to deep in the body, exploring whether bacteria there might influence the onset of asthma.

As odd as this might sound, there’s mounting evidence that bacteria matter. Babies born via cesar-

ean section, who experience a more sterile entry into the world than those born vaginally, are more likely to get asthma. So are young children treated with many courses of antibiotics. Along with animal studies, these observations suggest that the balance of bacteria and other microbes help guide immune development—and that when the balance is disrupted, disease may follow.

The picture can be dishearteningly complicated. Thousands of species of bacteria have constructed virtual cities inside us, along with fungi and viruses—a world called the microbiome. And it’s not so much the presence or absence of bacteria, or even certain species, that matter, but rather the shape of the whole community. All of us play host to bacterial residents. But children who develop asthma, researchers are learning, are home to different bacteria—and sometimes a less diverse mix—than those

who stay healthy. “It’s really coming down to the bacterial community structure, who’s there, and in what numbers, and where,” Huffnagle says. Cataloging these inhabitants is a new frontier.

Lungs and guts

For many years Hans Bisgaard, a pediatrician at the University of Copenhagen, was puzzled by a classic feature of asthma: Very young children with the disease have abundant neutrophils in their lungs, white blood cells that generally appear when the body is fighting infection. Given that asthma isn’t considered an infectious disease, this reaction seemed out of place.

Beginning in 2000, Bisgaard had a chance to assuage his curiosity. He and his colleagues were recruiting about 400 pregnant women with asthma to track the onset of disease in their offspring. They took throat

swabs from the babies when they were 4 weeks old and looked for certain pathogenic bacteria. To their great surprise, about a fifth harbored these microbes in their airways, including strains of influenza and pneumonia.

The newborns weren’t sick and so were left untreated. Five years later, 33% of those who had had pathogenic bacteria early on had asthma—compared with 10% of those without the bacteria. The work was published 3 years ago in *The*



Sterile birth. C-sections, which expose babies to fewer bacteria than vaginal births, are linked to a higher risk of asthma.

New England Journal of Medicine. “It’s redefined our course of research,” says Bisgaard. His team has since launched a second cohort study, this one of 800 pregnant women, most of them healthy, and has found the same prevalence of airway bacteria in newborns.

The work also upended how researchers think about lung biology. “If you read a medical textbook even now, it will say the lungs and the airways are sterile; there aren’t any bacteria down there,” says Cookson. He became certain that the conventional view was incorrect when he and an Imperial College colleague, geneticist Miriam Moffatt, conducted their own variation of Bisgaard’s study in babies. They had at their disposal advanced gene-sequencing techniques that allow for a much more comprehensive census of bacteria flourishing in the lungs. In January, the two and their colleagues wrote in *PLoS ONE* that they’d sequenced more than 5000 different species in 43 people, including some with asthma and others who were healthy.

The bacterial balance was quite different in the asthmatics: Affected children had more proteobacteria, which include pathogens like influenza. A different class—bacteria called bacteroidetes, found in soil, seawater, the gut, and skin—were more common in the nonasthmatics.

Cookson and Moffatt are now collaborating with Bisgaard to conduct gene sequencing of lung bacteria in Bisgaard’s Copenhagen cohort.

Similar discoveries may help explain the association between birth by C-section or abundant antibiotic use and increased risk of asthma in childhood. In Dutch research published in 2008, 8-year-olds born by C-section had nearly double the risk. Both C-sections and antibiotics modify the flora of gut and possibly lung bacteria, in some cases for many years. “The microbiota of children born through C-section is different; it’s less diverse,” and the same is true for antibiotic use, with fewer species present, says Anita Kozyrskyj, an epidemiologist at the University of Manitoba in Winnipeg, Canada.

And just as Cookson found different bacteria in the lungs of asthmatics, there’s growing evidence that this is true in the gut, too, says Fernando Martinez, a pediatric pulmon-

ologist at the University of Arizona, Tucson.

Researchers are probing the connection with animal work and following thousands of children from birth. A Canadian effort called CHILD is recruiting 5000 pregnant women and will track their children for at least 5 years. “We have lots of poop frozen away”—including the newborn’s first, right after birth—“dust from the babies’ beds, nasal swabs, [a] massive library of this information that we’ll be able to piece together,” says Stuart Turvey, a pediatric immunologist at the University of British Columbia in Vancouver, one of CHILD’s leaders.

So far, the evidence linking asthma and bacteria are associations, not proof that an imbalance of bacteria causes the disease. The big question, says Martinez, is, “Do asthmatic-



Hay protects. Youngsters on farms are less prone to asthma and allergies, but it’s not clear why.

ics have an immune system that makes them be colonized by different things? ... Or is it because they were colonized by different things that caused them to have asthma?”

From the inside out

Even less is known about what might be modifying lung and gut microbial communities and why children exposed to the same factors respond differently. The pattern isn’t clear-cut: Many children born vaginally, or who never got antibiotics, still develop asthma, just as many born by C-section or given antibiotics do not.

One piece of the puzzle is likely to be genetics: Some babies may be more prone to colonization by certain microbes, or to developing asthma once that colonization occurs. Another piece is the environment. For years it’s been known that children raised on farms are less likely to develop asthma and allergies than others. This phenomenon is often

referred to as the “hygiene hypothesis,” the idea that the relatively germ-free lifestyle most of us now lead can disrupt the development of the immune system.

In the countryside of five European countries, Erika von Mutius, a pediatrician and epidemiologist at Munich University Children’s Hospital in Germany, is sifting for clues. She and others are planning to sequence stool samples and throat swabs from more than 1100 children—half on farms and half in nonfarm rural settings. They’re also collecting samples of milk (farm milk is often unprocessed) and the components of dust for limited gene sequencing. “A lot of this is inhaled,” and because we’re talking about young children, “a lot of this is also probably ingested,” says von Mutius.

Although researchers assume that a child’s microbiome is affected by the environment, they don’t know this for sure. And proving definitively that bacteria help cause asthma is remarkably difficult. “The only proof lies in a randomized controlled trial, where you somehow manipulate exposure” and see who gets sick, says Bisgaard.

Researchers are experimenting with this approach in the gut. Probiotics, microorganisms like *Lactobacillus* found in yogurt, could in theory be helpful, but small trials testing whether they prevent allergic disease haven’t been definitive. In 2006, the University of California, San Francisco, began recruiting about 200 babies who have at least one parent with asthma. Half receive a probiotic and half get a placebo, and the researchers are focusing on early markers linked to asthma, like eczema and wheezing. In January, they reported that 6 months of probiotics in infancy did alter the balance of microbes in the babies’ guts, but final results are several years off.

However these studies turn out, there’s no question that asthma has diverse triggers. Huffnagle’s mice needed a series of insults—gut yeast, antibiotics, and mold—to cause an explosion of symptoms. If the bacterial communities living in children wind up high on the list of risk factors, that may help solve one of the biggest mysteries of all in asthma: why the number of cases has climbed so high, and how it might be pulled back down.

—JENNIFER COUZIN-FRANKEL



CONSERVATION BIOLOGY

The Fight for Yasuni

A group of scientists is on the verge of winning its battle to protect an Ecuadorian forest containing record biodiversity—but will the world pay to seal the innovative deal?

In 1997, 25 years after the first of many childhood canoe trips with his father down Ecuador's lower Napo River to the Limoncocha Biological Reserve, David Romo returned to find the site trashed beyond recognition. Once a pristine habitat fiercely defended by indigenous hunter-gatherers known as Waorani, the reserve had been overrun by a settlement of farmers who had moved in after construction of an oil-access road. The Waorani were gone, and so were many of the species that had made up the reserve's acclaimed biodiversity. Nowhere were once-common birds such as the grey-winged trumpeters and harpy eagles. The colonists had decimated the lake's fish and caiman populations, and poaching and tree-cutting felled the last primary forest in the area. "The forest was completely destroyed," Romo, a conservation biologist at Ecuador's San Francisco University of Quito, recounts with dismay.

Not long after Romo's unhappy return, the lure of oil threatened to bring similar devastation farther south to Ecuador's Yasuni National Park and the adjoining Waorani Ethnic Reserve. In 1989, this 17,000-kilometer section of the Amazon Basin had been designated a UNESCO Biosphere Reserve. But it also happens to sit atop Ecuador's second largest reserve of crude oil, a block of concessions collectively known as the Ishpingo Tambococha Tiputini (ITT) field. In 2003, that rich prize spurred plans to build a new oil-access road into the park, instilling fears

of a repeat of what happened in Limoncocha. "I realized then that we needed to bolster our conservation efforts with good, credible science if we were to have any chance of saving Yasuni," says Romo.

Over the past decade, he and more than 50 other biologists working in the area have documented Yasuni's remarkable biodiversity, providing evidence that its forest has the highest number of species on the planet, including an unprecedented core where there are overlapping world richness records for amphibians, reptiles, bats, and trees. And after helping to form a group called Scientists Concerned for Yasuni, Romo and his colleagues have waged an international campaign to protect the location.

This unabashed science-based advocacy has had an impact. In 2005, the year after the group published a preliminary analysis of Yasuni's biodiversity and recommended its protection, Ecuador's government rejected further road-building inside the park. Two years later, Ecuador President Rafael Correa went even further, offering a proposal in which his country would, in exchange for several billion dollars, keep the ITT oil permanently underground in order to protect the park and to fight global warming. Industrialized countries would essentially pay to keep the park's oil-

derived carbon in the ground.

After several years of political negotiations and drama, the innovative initiative took a significant step toward reality this summer when the United Nations agreed to oversee a trust fund paid to Ecuador for the project. If all goes according to plan, the initiative may serve as a model for preserving intact biodiversity in other oil-rich portions of the western Amazon. But that's a big "if": President Correa has vowed to allow drilling if the international community fails to compensate Ecuador sufficiently.

Finding "megadiversity"

The magnitude of Yasuni's species richness first became evident in 1992 when the Maxus oil company hired botanists to salvage plant specimens during construction of a new road entering the northwest section of the park, which borders Colombia and Peru. It was a thrilling and yet exceedingly difficult task, recalls Nigel Pitman, a tropical biologist at Duke University in Durham, North Carolina, who in 1999 completed an inventory of 1500 different species of trees. "Getting familiar with the trees can take years," says Pitman, "and even then it means making up your own names for the dozens of species that are new to science. ... It isn't even diversity any longer—it's hyperdiversity, or megadiversity."

But if field inventories were beginning to reveal the forest's extreme diversity, no one in

Online
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View a slide-
show of Yasuni's
diverse species.

Protected park. Beneath this forest canopy lies a record-setting number of species.

the 1990s was synthesizing the voluminous, unwieldy mass of independently conducted plot-based studies into an overall picture of Yasuni's biodiversity. "People came to this area to do their research and left," says Holger Kreft of the Georg-August University of Göttingen in Germany, who has found that among lowland forests, Yasuni holds the record for most species of epiphytes: plants that grow on plants. "There wasn't anything approaching a network of scientists."

That changed after 2003 when the Brazilian national oil company Petrobras announced plans for a new 54-kilometer road into an isolated section of the park that would give access to the ITT oil fields. It was already clear that the Maxus road had provided an entry point for people to colonize the forest and for illegal logging in the park's northwest section, says ecologist Matt Finer, whose Yasuni postdoctoral research quickly shifted to campaigning once he discovered little if any organized opposition to Petrobras's new plans.

To conservation biologist Margot Bass, the announcement by Petrobras, one of the largest companies in the world, seemed like an irrevocable death sentence for Yasuni park. "I despaired," says Bass, an executive director of Finding Species, a small environmental group, who had worked in the park in the late 1990s.

Nevertheless, she and Finer, now with the Washington, D.C.-based environmental group Save America's Forests, rallied together a who's who of leading tropical ecologists, organizing a 2-day conference in October 2004 at which attendees detailed the biodiversity significance of Yasuni and illustrated how the previous Maxus road had spurred deforestation. The researchers followed up with intense lobbying. "This was not pure science," says Luis Suarez, head of Conservation International's Ecuador office, who was not part of the group. "They decided to take a position and produce not only a scientific paper but also write letters, give presentations, and basically put Yasuni on the agenda."

Despite scientific and legal challenges, Petrobras began road construction and was on the verge of entering the park when, at the end of April 2005, President Lucio Gutierrez was deposed after massive protests of his plans to revamp Ecuador's Supreme Court. By July, the new government had revoked the oil company's permit for the road. Soon thereafter, President Correa stepped forward with his ITT proposal. "We kept so much heat on the issue that it gave bigger and better

funded organizations time to try and put together a really systematic plan for the region," says Bass.

A Copenhagen setback

In the 3 years since Correa floated the Yasuni trust fund plan, oil exploration has progressed nearby—in eastern Ecuador and across the border in southwest Colombia and northern Peru—and scientists working at Tiputini Biodiversity Station, one of two university-affiliated research stations in the park, say it's as if an unstoppable force is closing in. "Tiputini is still very remote," says Christian Voigt, an animal behavioral physiologist, "but you can hear the generator of an oil platform from a few kilometers' distance, and at night you can see the glow of the gas flame."

Yasuni's advocates have pressed on with amassing proof of its biodiversity riches, hoping such data will persuade the international



Make a deal. If paid billions of dollars, Ecuador will prevent oil exploration in Yasuni.

community to pay for the park's protection. In January, Bass, Finer, Kreft, Pitman, and their colleagues published a data-rich, collaborative analysis in *PLoS ONE* that confirmed the existence within Yasuni of a so-called quadruple richness center. This 28,000-km² plot encompasses peak species records for amphibians, birds, mammals, and tree communities. "Yasuni is probably unmatched by any other park in the world in total number of species. Both our species-distribution maps and our comprehensive analysis of existing field inventories support this finding," says Bass.

The area around the Tiputini station, for example, has smashed the world record for local amphibian diversity, with its 139 species far exceeding the 98 documented in Leticia, Colombia, the previous record-holder. And for insects, Yasuni's estimated 100,000 species per hectare represents the highest biodiversity, per unit area, in the world for any taxonomic group.

Those who have made Yasuni their research home, and their mission, expected that Correa's initiative would get off the ground at the Copenhagen climate summit last December. But the Ecuadorian government questioned whether it had enough authority over the trust fund, and no deal was struck then, infuriating the country's environmental and scientific communities. "There was, already, Germany committing \$50 million per year for 10 years," an angry Romo recalled this spring, hands waving in the air. "We had letters of intent from at least five other countries—and now you say you want to go to OPEC and ask them for the money. That's betrayal!"

Emotions have cooled since then, and in August Ecuador signed an agreement enacting the governance arrangements on a deal to keep Yasuni oil fields untapped in exchange for a minimum \$3.6 billion in payments (about half the value of the oil if sold) from industrialized countries over the next 13 years. The United Nations Development Programme, which will oversee the trust fund, has suggested that the agreement could serve as a model for protecting ecosystems around the world.

However, numerous questions about the effort remain, particularly in regards to the composition of the government-dominated board tasked with dispensing the trust fund's monies for conservation and reforestation projects, sustainable energy development, and livelihood-training programs for the local indigenous communities. There are fears, for example, that indigenous representatives will be excluded from the decision-making process.

The biggest uncertainty remains funding. Chile has already committed \$100,000, and Ecuador's vice president had said that Belgium, Spain, Turkey, and China have also offered money. But no official pledges from those countries have been announced, and in September, Germany signaled it was rethinking its vital commitment of nearly one-sixth of the needed total. If \$100 million isn't paid into the United Nations fund by December 2011, Ecuador can refund any contributions—and analysts say Correa will then surely move to develop Yasuni's oil fields.

Although he's nervous about whether the international community will fill the trust fund, Romo says he believes that the Yasuni researchers have so far succeeded in a way that cannot be ignored, providing justification for the region's continued conservation. "What caught the world's attention is the science," says Romo. "But the clock is running and we cannot get distracted." —ERIC MARX
Eric Marx is a freelance writer based in Berlin.

STEM CELLS

Diseases in a Dish Take Off

Reprogrammed cells from hundreds of patients are giving scientists the chance to model disease in new ways and are catching on among big pharma as well

Michael Venuti wants to rearrange the process of drug discovery. Usually patients enter at the last step, he says, in clinical trials. But as the CEO of iPierian, a biotech company in South San Francisco, Venuti is hoping to change that. Using reprogrammed stem cells taken from patients, iPierian and others are starting to develop lab models of disease that give researchers “a look at the patient in a dish,” he says. “Although we’re not putting patients themselves into the process, we are introducing them earlier than they have ever been included in the history of drug discovery.”

These advances are not the miracle cures some expect to come from stem cells: regenerated organs or a cure for diabetes. Such therapeutic advances are far in the future, experts agree. But ever since the first human embryonic stem cells were isolated, scientists have dreamed of using them to study intractable diseases.

That dream, says Kevin Eggan, a stem cell biologist at Harvard University, “is a reality. It is the early days of that reality.” There are still plenty of hurdles, but already researchers have shown that reprogrammed cells from patients with a handful of rare genetic disorders can provide new clues about how the diseases do their damage. In several cases, the cells have pointed toward promising new treatments.

The turning point came with the 2006 discovery that adult cells could be reprogrammed to an embryolike state simply by adding a handful of genes to the cells. The reprogrammed cells, called induced pluripotent stem (iPS) cells, have transformed the stem cell field. Not only do they get around many of the ethical issues that have dogged human embryonic stem cells, but they have also made it possible for researchers to make a ready supply of pluripotent cells from patients whose disease they are studying. (Pluripotent cells have the potential to become any cell type in the body.) The technology “made it so accessible for labs to get into the stem cell field,” says Gustavo Mostoslavsky, a stem cell scientist at Boston University School of Medicine. “For the

first time, we have the ability to take any disease that is out there, take a few cells from a patient, and create pluripotent cells in a dish,” he says. “I think we will see an explosion of papers in the next few years.”

Already, dozens of labs around the world are making iPS cells from patients and coaxing the cells to become the tissues that are affected by a given disease, for example, neurons from patients with amyotrophic lateral sclerosis (ALS). Researchers are comparing the ALS cells’ behavior with that of nondiseased cells to tease out what could be killing the patients’ neurons. Companies are using

fornia, and his colleagues reported that they had derived iPS cells from a patient with spinal muscular atrophy (SMA), a genetic disease that strikes children, often killing them by age 2. To develop a useful disease model, the researchers needed to prompt the iPS cells to make the affected tissue and then show that the cells exhibit characteristics of the disease in culture.

SMA was a promising candidate. Scientists knew how to grow motor neurons from embryonic stem cells, and they knew, in principle, how to cure the disease. (SMA has been called “the most curable neurological disease for which there are no treatments.”)

SMA patients have a faulty copy of a gene essential to the survival of motor neurons called *Survival Motor Neuron 1* (*SMN1*). Because their cells don’t make enough of the SMN1 protein, their motor neurons die and patients become paralyzed. People also have a gene called *SMN2* that makes the same protein but in much smaller amounts.

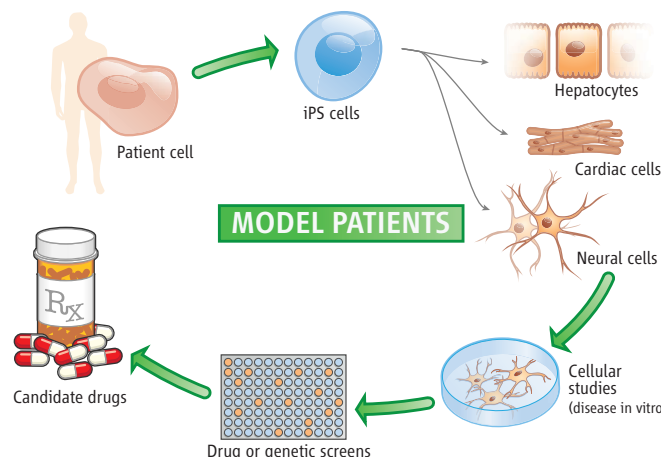
Some SMA patients have multiple copies of the *SMN2* gene, so they have milder symptoms and commonly survive into their 30s or 40s. The goal, then, is to find a drug that triggers the *SMN2* gene to make more protein.

Because mice and other common model animals lack the *SMN2* gene, it has been difficult to develop an animal model that truly reflects the human disease. And although scientists have used human skin fibroblasts to screen for compounds that boost SMN protein production, the cells

don’t seem to produce or use the protein in the same way as do motor neurons. With iPS cells, scientists had their first chance to study large quantities of motor neurons that carried the disease’s genetic defect.

As a control, Svendsen and his colleagues also made iPS cells from the SMA patient’s mother, who was unaffected by the disease. Initially, both cell lines behaved similarly, making healthy-looking motor neurons. But after a few weeks in culture, the SMA cells started to falter, with fewer and fewer neurons growing—the first sign that the cells reflected the disease phenotype. To see whether the cells would respond to potential drug candidates, scientists added two compounds known to boost SMN production. The model worked: The diseased cells produced up to three times as much SMN protein as their untreated counterparts.

Several groups are building on the work, screening hundreds of thousands of com-



Finding answers. Scientists are reprogramming cells from diseased patients, using them to better understand the disease process and search for new drugs.

heart cells derived from iPS cells to screen drugs for possible cardiac side effects—a common problem (see sidebar, p. 1173). Others are planning “virtual clinical trials” to test thousands of new drug candidates, using panels of dozens of cell lines from patients and from healthy controls.

The first successes in using iPS cells to study a disease in a dish have involved relatively rare genetic conditions that involve just a single gene malfunction. Nearly 2 years ago, at the University of Wisconsin, Madison, Clive Svendsen, now director of the Cedars-Sinai Regenerative Medicine Institute in Los Angeles, Cali-

Online

sciencemag.org

Podcast interview with author Gretchen Vogel.

pounds on Svendsen's cells as well as new lines from other SMA patients to search for possible drugs. iPierian is focusing on SMA, and early screens have been promising, Venuti says, turning up compounds that seem to work preferentially in motor neurons.

Another example is the rare genetic disorder called familial dysautonomia (FD). Developmental biologist Lorenz Studer at the Sloan-Kettering Institute in New York City and his colleagues derived iPS cells from patients with FD, a disease in which the peripheral neurons degenerate. The researchers described in *Nature* in September 2009 how they prompted the FD iPS cells to form neural crest precursor cells, known to be affected by the disease. (The neural crest forms in early development and gives rise to facial bones, smooth muscle, peripheral nerves, and other tissues.) The cells from FD patients expressed significantly lower levels of the genes involved in neuronal development and formed fewer neurons than control cells. These cells, too, passed the crucial disease-model test: When the scientists treated the cells with kinetin, a plant hormone known to affect the gene implicated in FD, the FD cells made more of the missing protein and formed more neurons. Exposing cells to the hormone for several weeks had the greatest effect, an observation that

could be important to the design of early clinical trials of kinetin in FD patients, Studer says. Studer and colleagues have developed ways to make hundreds of millions of cells, with a robot distributing them onto the test plates, and are using them to screen thousands of compounds. The scientists are also comparing cells from patients with early- and late-onset disease to see if they can understand the delay in symptoms. "Studer is really pushing the field forward," says Eggan.

Some genetic syndromes may help scientists understand much more common diseases as well. This month, Alysson Muotri, a neurobiologist at the University of California, San Diego, and his colleagues reported in *Cell* that iPS cells from patients with Rett syndrome form neurons that have multiple defects compared with control cells. Rett patients develop normally for 6 to

18 months, but then lose muscle control, develop seizures, and show increasingly autistic behavior. Muotri and his colleagues hope that the model could help scientists to understand better what goes wrong in other autism spectrum disorders.

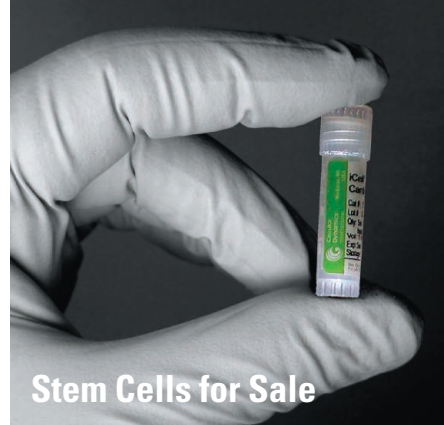
Several groups have made iPS cells from patients with genetic heart defects, including long QT syndrome, which can cause deadly arrhythmia, and LEOPARD syndrome, a disorder involving thickened heart muscle and other defects. The cells are far from a perfect model of more common heart disease, says developmental biologist Christine Mummery of Leiden University Medical Center in the Netherlands. But given the dearth of animal models for that major killer, the cells are a promising step, she says.

Dozens of labs are working with iPS cells from patients with more common conditions, including Parkinson's, ALS, schizophrenia, and Alzheimer's. But those diseases will be tougher to study. They result from a mix of genetic and environmental factors, and it is not yet clear how well iPS cells will reflect the environmental history of the cell donor. Still, the chance to work with unlimited quantities of cells derived from patients with these diseases is unprecedented. Until recently, Eggan says, "we haven't had a way to get at diseases that are sporadic but obviously have a genetic component."

Stem cell researcher James Thomson of the University of Wisconsin, Madison, cautions that researchers don't yet fully understand the variation in iPS lines. If two lines behave differently, it is difficult to tell whether that is because of disease-relevant genetic differences or random differences introduced in the reprogramming process, although newer techniques are helping. Studer agrees. "The big question in the field is variability" in reprogrammed cells, he says.

Despite the challenges, more diseases are going to yield their secrets using iPS cells, Eggan predicts. "It's going to happen. It's just hard work." Considering that reprogramming technologies are less than 5 years old, he says, "it's hard not to be pretty pleased with the progress that's been made in a few short years."

—GRETCHEN VOGEL



Stem Cells for Sale

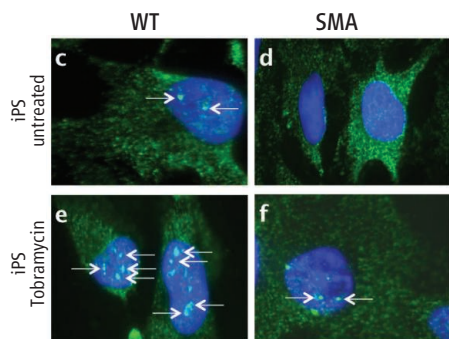
Dozens of biotech companies hope to use stem cell technologies to make products that turn a profit, but very few have. Cellular Dynamics International (CDI), based in Madison, Wisconsin, is an exception. CDI sells cardiomyocytes—heart muscle cells—derived from induced pluripotent stem (iPS) cells for \$1500 per vial. Each vial contains about 1.5 million frozen cells, enough to fill a 96-well plate with beating heart cells that survive longer than 2 weeks in culture, says Chris Parker, CDI vice president and chief commercial officer.

There are plenty of buyers. Parker says several dozen pharmaceutical companies are already using the cells to screen new drugs for side effects that affect the heart, one of the most common reasons otherwise promising candidates fail.

The standard tests for these side effects use animal cells, cancer cells with an introduced heart gene, and dogs. "It takes roughly \$2 billion and 10 years to get a drug to market—and despite that we are still getting drugs on the market with cardiac side effects" that later have to be withdrawn, says Christine Mummery, a developmental biologist at Leiden University Medical Center in the Netherlands. She and her colleagues were some of the first to demonstrate that cardiomyocytes derived from human embryonic stem (ES) cells could predict a drug's side effects.

Cardiomyocytes often form spontaneously in human ES cells or iPS cell cultures that are left to differentiate on their own. But getting large quantities of pure, mature cells has been a challenge. CDI's scientists have managed to find ways that prompt industrial-scale quantities of iPS cells to develop preferentially into heart cells. They also developed a cell-sorting technique that results, Parker claims, in a cell population that is "99% cardiomyocytes." Cardiomyocytes don't divide, so the cells are a "consumable product," he says. The company guarantees that they will beat in culture dishes for at least 2 weeks. "So far so good," Parker says. "We haven't had anyone send their vials back."

—G.V.



Proof of principle. Neurons derived from patients with spinal muscular atrophy (right panels) increase the production of a key protein when treated with a potential drug (bottom panels).



Empowered. Locals are included in an archaeological excavation in Lesotho.

Archaeologists, or Activists?

When Charlie Arthur started his archaeological work in the small southern African country of Lesotho in 2008, the clock was already ticking. The Phuthiatsana River valley is set to be flooded by a dam in 2012, submerging a rich record that includes Paleolithic tools and beautiful rock art. If this dig was going to be like previous ones in the region, the University of Oxford archaeologist would have zipped in and out with a minimum of interaction with local people and hauled the artifacts back to the United Kingdom. But despite the time pressure, that's not what happened.

First, Arthur's team invited schoolchildren to learn about the ancient tools and rock art in their neighborhood. They trained locals, who were soon maintaining sites on their own. They arranged to donate equipment to local institutions and started a newsletter in the native language. They even appeared on television. Without this approach, says Arthur, "no benefit at all would come to the local heritage structures or communities under threat from the dam."

The Lesotho project was one of dozens involving engaged archaeology presented at Cheikh Anta Diop University here earlier this

month. The Society for Africanist Archaeologists met for the first time in Africa instead of Europe or the United States, joining forces with the Congress of the PanAfrican Archaeological Association (PANAF). The older generation beamed with pride. "Fifty years ago, many scholars thought that Africa was at best a trivial pursuit," says archaeologist Merrick Posnansky of the University of California, Los Angeles. Now "African nationals are learning about their own past."

But the new generation is inheriting a troubled field. As Africa is rapidly transformed by development, archaeological sites are being destroyed faster than they can be studied. At the same time, there are "contrasting priorities between African nationals and foreign scholars," says Posnansky. Rather than publishing papers, archaeologists who grew up on the impoverished continent are often motivated by the need to create jobs for locals and boost national or ethnic pride.

"I joined archaeology as an activist," says PANAF president Alinah Segobye, based at the University of Botswana in Gaborone. In the traditional view, archaeologists should be objective scientists who generate knowledge about the past and steer clear of politics. But for Segobye, "archaeology has been a tool to engage with development, focusing on such issues as the fight against poverty." The



**Early Dates
For Diepkloof**

For the researchers who study the famed Diepkloof rock shelter in South Africa, the meeting was both a coming-out party and a defense. Diepkloof is a key Paleolithic site, the home of 60,000-year-old decorated ostrich shells that are among the first signs of symbolism. Now the team suggests that sophisticated behavior there stretches back as far as 130,000 years ago—nearly twice as old as at any other site.

Guillaume Porraz of the University of Tübingen in Germany presented the first "grand synthesis" of 15 years of work at the site. On a screen behind him was a timeline of data, including an analysis of thousands of stone tools, plant and animal remains, and cooking hearths. In front of him, the international gathering of archaeologists collectively

Mystery. Are these stone tools (*inset*) from Diepkloof the oldest signs of modern culture?

raised their eyebrows: He seemed to gain few immediate converts.

The first signs of "modern" human behavior appear in southern Africa, in two bursts of innovative technologies. The first, called Stillbay, includes long, sharp blades that required complex planning to make (*Science*, 29 October, p. 659). Next came an even more sophisticated phase called Howieson's Poort, which includes the engraved ostrich eggs as well as highly standardized blades and points that were glued into spears, allowing long-range attack.

Stillbay was a very brief period, possibly as short as a millennium, that ended about 71,000 years ago, according to previous work by a team led by Zenobia Jacobs of Australia's University of Wollongong (*Science*, 31 October 2008, p. 733). She used optically stimulated luminescence to date sediment from sites across southern Africa, including Diep-

CREDITS (TOP TO BOTTOM): JESS MEYER, P-J TEXIER, DIEPKLOOF PROJECT (2)

notion that archaeologists can do their work “in a vacuum” is a myth, says Gerry Wait, director of Nexus Heritage in Fordingbridge, U.K., who gave a talk on the issue. “Archaeology is in the realm of political action.” Wait called on his colleagues to replace archaeology’s ethical code of “do no harm” with the activist aim of “doing good.”

Considering the audience, he was mostly preaching to the choir. But some voiced caution. “There can be tension between doing no harm and doing good,” says archaeologist Willem Willems of the University of Leiden in the Netherlands, especially when scientific evidence is ignored for the sake of political goals.

Africa has such cases, says Arthur. In Zimbabwe in the 1990s, archaeologists helped the government reconstruct the 19th century capital of the precolonial kingdom near Bulawayo. The evidence showed that the main building had an east-facing entrance. But east-facing entrances are taboo to tribes that now live there, so “the house was reconstructed with the door facing the wrong way.”

Such compromises may be a small price to pay to help Africans embrace archaeology, says Arthur. Nonetheless, he’s uncomfortable wearing the “activist” mantle, preferring the more research-oriented “community archaeology” (*Science*, 26 August 2005, p. 1317). “When major sites are threatened, they still need digging and recording so that this information is not lost.” Whatever material his team can save will go into the country’s first national museum.

—J.B.

kloof, and dated Howieson’s Poort to between 65,000 and 60,000 years ago. Between the two periods, people reverted to simpler tools.

The Diepkloof team found much older dates using thermal luminescence, which dates burnt stone instead of sediment. Those results, published last year, pushed the start of the Stillbay period back to 130,000 years ago and its end to 92,000 years ago. The team also says the Howieson’s Poort period began 87,000 years ago and ended 55,000 years ago. Their new sediment analyses show that Jacobs’s dates were based on erroneous assumptions, they say.

Most archaeologists who spoke with *Science* were skeptical. If the new dates are right, says Michael Chazan of the University of Toronto in Canada, “it throws the [African] sequence out of whack.” But the jury is out until the Diepkloof team publishes its new analysis.

—J.B.



Odyssey. Livingstone Smith (squatting at right) digs for artifacts along the Congo River.

Latter-Day Livingstone Digs Along the Congo

It was like a scene from *Heart of Darkness*, but with a happy ending and exciting scientific results. A wiry, bearded Belgian named Alexandre Livingstone Smith recounted his recent journey down the Congo River. Archaeologically, the sediment beneath the lush central African jungle is “unexplored territory,” says Livingstone Smith, an archaeologist at the Royal Museum for Central Africa (RMCA) in Tervuren, Belgium. To perform the first archaeological survey of the center of the continent, he hitched a ride with a team of biologists doing a biodiversity survey from April to June.

Archaeologists have a detailed picture of human activity on each side of Africa, such as the early ironworking culture around Lake Victoria to the east and the complex pottery-producing societies of Cameroon to the west. Yet the connection between the two regions in the Congo River basin remains a blank spot. Parasitic diseases, inaccessibility, thick underbrush, and constant rain all make archaeological work in the rainforest difficult, but the main problem is the region’s lawless violence. “When you excavate a long time at the same place, you become a target for well-planned attacks,” explains Livingstone Smith. Quick surveys of clearings along riverbanks are safest.

So that’s what the latter-day Livingstone did on his 500-kilometer journey. (Ironically, he may be a distant relative of David Livingstone, the legendary Scottish explorer who mapped the waterways of the former Belgian colony.) He joined the expedition at Kisangani, replacing his RMCA colleague Els Cornelissen on the boat, and traveled to Bumba, on a trip that was no leisure cruise.

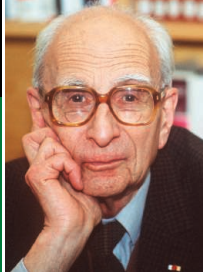
“The biologists would only stop for a day or two at any one place,” he says, “enough time to capture bugs and sample leaves.” For archaeology, that is a frenzied schedule, and meticulous excavation was out of the question. So Livingstone Smith surveyed rapidly, digging shallow test pits, and going deeper only at the most promising sites.

The banks of the Congo were chock-full of archaeological material. Livingstone Smith and Cornelissen identified dozens of occupation sites containing everything from stone tools to pottery. They bagged samples of burnt charcoal and nuts to carbon-date back in the lab. The best site was an ancient burial tomb pointed out by a woman from a nearby village. “It was near the edge of a cliff and it turned out to be very deep,” he says. Inside were carefully arranged layers of pots, including one that seemed designed to hold a child’s body. But like elsewhere, there were no traces of human remains. “The acidity of the soil dissolves all bone.”

The design of some of the pottery—ranging from small cups to storage vessels with beveled rims and comb-pressed stamping patterns—is unlike any so far described. These could be a “missing link between east and west” Africa, says Livingstone Smith. Another style of pottery, called Imbonga and dated to roughly 3000 years ago, had been previously found in the region. But Livingstone Smith found Imbonga pots on a different river, 1000 kilometers east of their previously known location. That helps confirm the idea that people settled the region by river, he says.

The results show that this kind of “broad-spectrum reconnaissance” is still valuable, says archaeologist Susan Pfeiffer of the University of Toronto in Canada. But a deeper understanding of the ancient people of the Congo River Basin will have to wait for government stability.

—JOHN BOHANNON



LETTERS

edited by Jennifer Sills

Make Way for Ethanol

IN HIS NEWS STORY "IS THERE A ROAD AHEAD FOR CELLULOSIC ETHANOL?" (SPECIAL SECTION ON Scaling Up Alternative Energy, 13 August, p. 784), R. F. Service identifies factors contributing to fading enthusiasm for cellulosic ethanol and observes that policy-makers' decisions this year could shape the nascent U.S. biofuels industry for decades. It is critical at this time to distinguish the fundamental from the ephemeral and to base policy on the former rather than the latter.

Biomass is by far the most viable sustainable source of liquid fuels, which will be needed for a long time, if not indefinitely. Batteries are completely impractical for aviation and likely for long-haul trucks as well. In the most aggressive scenarios for electrification of light-duty vehicles, liquid fuels still provide more than 50% of U.S. transportation energy (1). Policy debates are often framed as if large-scale use of biofuels is discretionary, but achieving a sustainable transportation sector is much more likely with biofuels than without them, and turning away from biofuels entails substantial risks.

The most important next step in the biofuels arena is commercial production of ethanol from cellulosic feedstocks. The alternative—converting sources of readily fermentable sugars (mostly from corn and sugar cane) to fuel

molecules other than ethanol—might allow us to retain current infrastructure, but would contribute little to our larger goals: creating a sustainable energy supply, reducing greenhouse gas emissions, assuring energy security, and promoting rural economic development. Ethanol will very likely be the world's first cellulosic biofuel because it is unrealistic to commercialize new technology for converting lignocellulose to sugars at the same time as new technology for converting sugars to fuels. Infrastructural challenges associated with distribution and utilization of ethanol are readily solvable, as the Brazilian experience shows, and decidedly small compared to challenges associated with other petroleum alternatives such as batteries or hydrogen.

Sharply divergent conclusions have been drawn about biofuel-related land-use issues, contributing to uncertainty among policy-makers. Pessimists typically ask: What are the impacts of adding large-scale use of today's version of biofuels to the world that will exist if current trends and practices continue? Optimists typically ask: What role could the advanced biofuels of the future play in a world reconfigured to meet energy-related challenges? Both questions merit consideration, but only the second illuminates a promising way forward. There is increasing evidence that large-scale biofuel production can be gracefully reconciled with feeding humanity and preserving the environment, provided that changes are made pursuant to this goal. Change is necessary, given that humanity cannot hope to achieve a sustainable and secure future by continuing the practices that have led to the unsustainable and insecure present.

Most of the factors mentioned in Service's News story are peculiar to the United States. Brazil, for example, did not experience the recent economic downturn, does not have a "blend-wall" (ethanol production approaching the maximum amount that can be accommodated in

10% gasoline blends) or policy uncertainty relative to bioethanol, and is confident of the sustainability of its land-use practices. Ethanol is produced in volumes exceeding gasoline usage (2) from about 1.5% of Brazil's arable land, and the cane used to produce this fuel is grown on former pastureland while total pasture output has increased (3). The international community should be careful not to draw negative conclusions from the U.S. malaise, particularly in light of the high stakes involved.

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References and Notes

1. Light duty vehicles (LDVs) account for 59% of total U.S. transportation energy (4). Of the total miles driven in LDVs, about 70% are in vehicles that drive less than 60 miles per day (5). If half of all LDVs were dedicated electric vehicles and the other half were plug-in hybrid electric vehicles that used batteries for the first 60 miles driven per day—a more aggressive electrification scenario than any proposed thus far—the fraction of transportation energy provided by electricity would still be only half [(0.5 + 0.5 × 0.7) × 0.59].
2. National Agency of Petroleum, Natural Gas and Biofuels, "Oil, natural gas, and biofuels statistical yearbook - 2010" (Ministry of Mining and Energy, Rio de Janeiro, 2010), Table 4.9; www.anp.gov.br/?dw=31814.
3. J. Goldemberg, S. T. Coelho, P. Guardabassi, *Energy Pol.* **36**, 2086 (2008).
4. U.S. Department of Energy, *Transportation Energy Data Book* (Center for Transportation Analysis, Knoxville, TN, ed. 29, 2010); www.cta.ornl.gov/data/index.shtml.
5. U.S. Department of Transportation, "2010 national household travel survey" (Oak Ridge National Laboratory, Oak Ridge, TN, 2010); <http://nhts.ornl.gov/tools.shtml>.

Permafrost and Wetland Carbon Stocks

IN THEIR REPORT "GLOBAL CONVERGENCE in the temperature sensitivity of respiration at ecosystem level" (13 August, p. 838), M. D. Mahecha *et al.* have done a fine study



Sugar cane in Brazil.

to tease apart the distinction between intrinsic and apparent temperature sensitivities (1). However, they omitted the soil carbon stocks most vulnerable to climate change—northern permafrost and most wetlands were excluded from the data (2).

In his related Perspective “The carbon dioxide exchange” (13 August, p. 774), P. B. Reich concludes that Mahecha *et al.*’s study “reduces fears” that biotic feedbacks to climate change will amplify the effects of temperature increase. Yet Mahecha *et al.*’s analysis of data from mostly upland forests, grasslands, and croplands cannot represent the temperature sensitivity of decomposition during the phase change from frozen to liquid water in thawing permafrost (3). Similarly, large stocks of relatively labile carbon become exposed to aerobic decomposition

when wetlands drain, and this climate sensitivity cannot be modeled with data dominated by upland ecosystems.

When carbon stocks hitherto protected by frozen or anaerobic conditions become vulnerable to high rates of decomposition due to climate change, temperature will be important. I urge readers not to be lulled into a false sense of security by misinterpretations of Mahecha *et al.*’s results. **ERIC A. DAVIDSON**

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References

1. E. A. Davidson, I. A. Janssens, *Nature* **440**, 165 (2006).
2. C. Tarnocai, *Global Biogeochem. Cyc.* **23**, GB2023 (2009).
3. C. J. Mikan, J. P. Schimel, A. P. Doyle, *Soil Biol. Biochem.* **34**, 1785 (2002).



LIFE IN SCIENCE

Lab Family Feud

I had just returned from a year-long sabbatical abroad. Refreshed, I looked forward to beginning another productive year. My plans were soon derailed. In my absence, the social fabric of my lab had deteriorated to a point where squabbling, backstabbing, and even screaming were common. My response to this turmoil was indecisive at best. After one particularly painful encounter, it dawned on me that I was in way over my head.

In desperation, I turned to the head of my university’s human resources office, who was a trained family therapist. After talking to her individually, she suggested group sessions with all of the lab members. After just one session, she gave us her diagnosis: We were a dysfunctional family, complete with rebellious adolescents (senior students), impressionable children (junior students), and impotent parents (technicians and the principal investigator).

I don’t know if it was the shock of forced group therapy or the skill of the therapist, but the sessions worked. The students and technicians relearned how to live and work together, the new students in the lab weren’t exposed to continued tension, and I regained control of the lab.

Years in science had done little to prepare me for the psychological challenges of running a lab. I’ve since recommended the use of an organizational psychologist to several other lab heads. A few hours of concentrated work can go a long way toward resolving interpersonal problems, and more productive science is sure to follow.

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EDITOR’S NOTE

This is an occasional feature highlighting some of the day-to-day humorous realities that face our readers. Can you top this? Submit your best stories at www.submit2science.org.

Response

I APPLAUD DAVIDSON FOR REMINDING US TO be careful of how general statements may be perceived, both in and out of context. Mahecha *et al.*’s conclusions were based on analyses of upland ecosystems, primarily forests and grasslands. When I wrote that their work “reduces fears that respiration fluxes may increase strongly with temperature, accelerating climate change,” I intended my interpretation to be applied only within the narrow constraints of the study conditions.

It is true that such conclusions cannot be applied to long-term responses of wetlands and permafrost, where much belowground carbon resides globally. Mahecha *et al.*’s conclusion that the medium-term sensitivity of ecosystem respiration to temperature may be modest could greatly underpredict carbon losses as permafrost thaws or wetlands drain, and it would be an illogical way to model processes involving large, episodic disturbances.

In the long term, changes in amounts and temporal patterns of precipitation may lead to more droughts and floods. Climate change can also indirectly influence terrestrial ecosystem structure and function, resulting in more fires, windstorms, and disease and pest outbreaks. If these direct or indirect climate effects lead to structural breakdown of existing communities (which often occur with floods, severe droughts, forest dieback, or wildfires) or slower but substantive shifts in structure as communities metamorphosize, carbon losses from decomposition and combustion would spike upward, too. Applying short-term analysis of ecosystem respiration by temperature would not serve as an accurate metric for modeling such processes either.

Davidson provides a valuable reminder that we need to maintain a holistic, long-term, and global perspective on biotic feedbacks to the global carbon cycle.

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Letters to the Editor

Letters (~300 words) discuss material published in *Science* in the previous 3 months or issues of general interest. They can be submitted through the Web (www.submit2science.org) or by regular mail (1200 New York Ave., NW, Washington, DC 20005, USA). Letters are not acknowledged upon receipt, nor are authors generally consulted before publication. Whether published in full or in part, letters are subject to editing for clarity and space.

CLIMATE CHANGE

Fixing the Planet?

Kevin E. Trenberth

The Intergovernmental Panel on Climate Change (IPCC) reported in 2007 that global warming is unequivocal and very likely caused by human activities, mainly through increasing carbon dioxide and other greenhouse gases in the atmosphere. Projections suggest future climatic changes at rates that are apt to have major disruptive impacts on societies and the environment. To date, politicians around the world have failed to adequately deal with this major threat. Possible ways forward assessed by the IPCC include “mitigation” through slowing emissions of greenhouse gases and “adaptation” through taking steps that “might ultimately enhance resilience or reduce vulnerability to observed or expected changes in climate” (1). One proposed solution is “geo-engineering,” and Eli Kintisch’s *Hack the Planet* examines the prospects of and past attempts at this tactic. Roger Pielke Jr.’s *The Climate Fix* proposes a different approach, one based on decarbonizing the economy and devoting greater efforts to adaptation.

In its series of four major reports since 1990, the IPCC has performed comprehensive assessments of the science of climate change, adaptation, and mitigation options in a policy-relevant but not policy-prescriptive way—at least in principle. IPCC participants come from scores of countries and all political persuasions and, in my experience, have always striven to provide the best science appraisal possible. They have produced a consensus but conservative set of documents. Although the Kyoto Protocol was a first step in addressing the problem politically, lack of further success has led Pielke (a political scientist at the University of Colorado) to slam the scientists, the IPCC, and politicians and to suggest rethinking our approach to climate change and climate policy. Kintisch (a reporter for *Science*) is much more accepting of the science and makes a case that the uncertainties are actually a call for action.

On the heels of the major failures of the December 2009 United Nations Copenhagen conference and the lack of any action in the U.S. Senate, Pielke outlines his view of these results and the factors involved. He highlights the focus on the importance of

carbon dioxide. Kintisch goes further and notes the critical impact of burning coal. Neither author adequately addresses the issue of how what is done gets done. Politicians and other decision-makers must embrace the role of enabling sensible planning and implementation of actions to avoid disruption. For instance, we need a climate information system to inform decisions and to feed into a climate service (2).

Certain sections of Pielke’s book contain a lot of spin. For instance, in his discussion of tradeoffs between the economy and the environment, he offers an “iron law of climate policy”: “when environmental and economic objectives are placed into opposition with one another in public or political forums, it is the economic goals that win out.” An example that he might have mentioned, but does not, is President George W. Bush’s 2001 rejection of the Kyoto Protocol on the grounds that it would hurt the economy. In considering the scope of this law, however, Pielke treats economic and environmental gains as mutually exclusive. Although his law may hold when that premise is accepted, Pielke does not acknowledge that the imposition of a price on carbon emissions can be offset with reductions in taxes elsewhere and made revenue neutral. Nor does he allow for innovative implementation strategies (e.g., increases in efficiency to offset added initial costs) that remove the head-to-head conflict between environmental and economic gains. By painting the issue as black and white, Pielke reaches flawed conclusions.

Other issues are mischaracterized and overplayed by Pielke, such as the minor errors in the 2007 IPCC report and the role of e-mails stolen from the University of East Anglia’s Climatic Research Unit (dubbed “climategate”). Although climategate has proven a major setback to climate science policy, that is not because there was any real substance to the charges (other than the way in which the United Kingdom’s freedom of informa-

tion act was abused)—see the independent panel reviews provided in the Oxburgh and Muir Russell reports (3, 4). Kintisch indeed dismisses climategate as having no effect on the validity of the science.

In his discussions, Pielke presents case studies for decarbonizing the economies of different countries and the unrealistic nature of many targets in isolation. The absence of an international framework means a country going it alone is potentially disadvantaged in the international marketplace relative to countries that exploit cheaper subsidized fossil fuels at the expense of climate change. But Pielke does not address the international lobbying for economic advantage inherent in the policy negotiations.

Pielke makes a big deal about differences in the definitions of climate change used by the United Nations Framework Convention on Climate Change and the IPCC. The former defines the topic narrowly in terms of human influences, whereas the latter defines it to be climate change from all causes. Although many of us would agree that more attention is warranted to adaptation and planning for future climate changes, his logic about IPCC bias against adaptation is contorted:

He objects to Working Group III’s favoring of mitigation (which is, after all, its mission) while ignoring Working Group II (whose mission is adaptation). His claims that “the science of climate change becomes irrevocably politicized” because “[s]cience that suggested large climatic impacts on Russia was used to support arguments for Russia’s participation in the [Kyoto] protocol”—as if there would be no such impacts and Russia would be a “winner”—look downright silly given the record-breaking drought, heat waves, and wildfires in Russia this past summer.

Unlike Kintisch, Pielke evidently does not appreciate how climate change is manifested mainly through changes in extremes. If a region undergoes only a modest shift in climate, the weather most of the time will fall within the same range experienced prior to the change. (For example, when probability distribution curves for temperature differ only by a small change in the mean value, they share nearly all of the area under them.) Pielke’s discussion of changes in extremes (as detailed in the chapter “Disasters, Death, and Destruction”) can only be described as

The Climate Fix
What Scientists and
Politicians Won’t Tell You
About Global Warming

by Roger Pielke Jr.

Basic Books (Perseus Books Group), New York, 2010.

288 pp. \$26, C\$32.95, £15.99.
ISBN 9780465020522.

Hack the Planet
Science’s Best Hope—
or Worst Nightmare—
for Averting Climate
Catastrophe

by Eli Kintisch

Wiley, Hoboken, NJ, 2010.

285 pp. \$25.95, C\$30.95,
£17.99, €20.80.
ISBN 9780470524268.

The reviewer is at the National Center for Atmospheric Research, Post Office Box 3000, Boulder, CO 80307, USA.
E-mail: trenbert@ucar.edu

a diatribe against the IPCC and other scientists that is based on highly selective and distorted figures and his own studies.

Pielke stresses economic data and dismisses the importance of loss of life. (For instance, he gives nary a mention of the over 50,000 lives lost to heat stress during the summer 2003 heat waves in Europe, which are discussed by Kintisch.) He makes “corrections” for some things (notably, more people putting themselves in harm’s way) but not others. Some adjustments, such as for hurricane losses for the early 20th century, in which the dollar value goes up several hundred-fold, are highly flawed. But he then uses this record to suggest that the resulting absence of trends in damage costs represents the lack of evidence of a climate component. His record fails to consider all tropical storms and instead focuses only on the rare land-falling ones, which cause highly variable damage depending on where they hit. He completely ignores the benefits from improvements in hurricane warning times, changes in building codes, and other factors that have been important in reducing losses. Nor does he give any consideration to our understanding of the physics of hurricanes and evidence for changes such as the 2005 season, which broke records in so many ways (5).

Similarly, in discussing floods, Pielke fails to acknowledge that many governing bodies (especially local councils) and government agencies (such as the U.S. Army Corps of Engineers) have tackled the mission of preventing floods by building infrastructure. Thus even though heavy rains have increased disproportionately in many places around the world (thereby increasing the risk of floods), the inundations may have been avoided. In developing countries, however, such flooding has been realized, as seen for instance this year in Pakistan, China, and India. Other tenuous claims abound, and Pielke cherry-picks points to fit his arguments. For example, a box titled “U.S. Extreme Events: Excerpts from a 2008 U.S. Government Report” presents six points related to changing extremes, but Pielke’s biased selection of those particular points from the numerous ones included in the report (6) completely changes the actual conclusions.

Pielke believes that there has been “systematic misrepresentation of the science of disasters and climate change ... in the leading scientific assessments produced to inform policy,” an action that he blames on “politi-



cal dynamics.” Only by dismissing any role of climate change in the increasing frequency or magnitude of disasters around the world is he able to make such outlandish claims. He goes on to say that “using disasters to advocate for mitigation policies is misguided at best and misleading at worst” and has led to “some of the most egregious errors in leading scientific assessments of climate change.” Pielke’s faulty premise drives his subsequent arguments. His utter failure to adequately appreciate climate science and the implications of its projections for the future means that *The Climate Fix* falls far short of adequately addressing major issues. Although progressively decarbonizing the economy and adopting an approach of building more resiliency to climate events would be good steps in the right direction, they are not enough. Planned adaptation to climate change may work for modest changes, but over 50 years from now, one form of adaptation will have to be to suffer the consequences—as, in fact, we are already doing. Or is geoengineering the answer?

Whereas Pielke’s account tends to minimize the risks associated with climate change, Kintisch’s very readable book emphasizes them. Each chapter opens with an example of past human attempts to forge geoengineering solutions to various kinds of problems, few of which were successful. The essence of geoengineering is not to attempt to directly forestall the problem but rather to implement alternative devices to deal with the symptoms. The difficulty with this approach is that even when it proves possible to alleviate the immediate predicament, there are often unintended consequences and side effects that could prove even worse than the original problem. This is certainly a major worry in regards to some suggested geoengineering fixes of global warming. For instance, emulating

volcanoes by injecting a veil of aerosol into the stratosphere may well stem (or at least reduce) increases in global mean temperatures, but doing so could also cause major droughts or other undesired regional effects (7). After all, global mean temperature is simply one indicator of climate change and does not reflect most aspects of the threat.

Moreover, the bandage approach has substantial costs and diverts attention away from a lasting solution to the problems caused by our transfer of carbon from fossil fuels into the atmosphere. It also raises major ethical questions concerning who is entitled to make the decision (on behalf of all humanity) to intentionally change the climate—as opposed to the unintentional changes we are making at present. In addition to exploring the issues and difficulties (including legal entanglements and the legitimacy of carbon offsets), Kintisch provides vignettes of several scientists active in geoengineering.

Geoengineering is also dealt with by Pielke, but only briefly. He makes a reasonable case that the level of uncertainty and ignorance with regard to climate change and the risk of unintended consequences limit its prospects. He suggests that “carbon remediation” (by which he means removing carbon dioxide from the atmosphere) may be feasible. However, he does not address the practicality of storing all of the carbon dioxide. Both carbon remediation and carbon sequestration are considered much more thoroughly by Kintisch.

Unfortunately, *The Climate Fix* often toys with the truth and uses highly selective evidence to bolster its case, further politicizing climate change science. Readers of *Hack the Planet* will gain a better understanding of the severe challenges posed by anthropogenic changes to Earth’s climate.

References

1. www.ipcc.ch/pdf/assessment-report/ar4/wg2/ar4-wg2-chapter17.pdf.
2. K. E. Trenberth, *WMO Bull.* **57**, 17 (2008).
3. www.uea.ac.uk/mac/comm/media/press/CRUstatements/SAP.
4. [www.cce-review.org/pdf/FINAL REPORT.pdf](http://www.cce-review.org/pdf/FINAL%20REPORT.pdf).
5. R. A. Anthes et al., *Bull. Am. Meteorol. Soc.* **87**, 623 (2006).
6. T. R. Karl et al., Eds., “Weather and climate extremes in a changing climate. Regions of focus: North America, Hawaii, Caribbean, and U.S. Pacific Islands” (U.S. Climate Change Science Program, Department of Commerce, Washington, DC, 2008); <http://downloads.climate-science.gov/sap/sap3-3/sap3-3-final-all.pdf>.
7. K. E. Trenberth, A. Dai, *Geophys. Res. Lett.* **34**, L15702 (2007).

10.1126/science.1197874

ANTHROPOLOGY

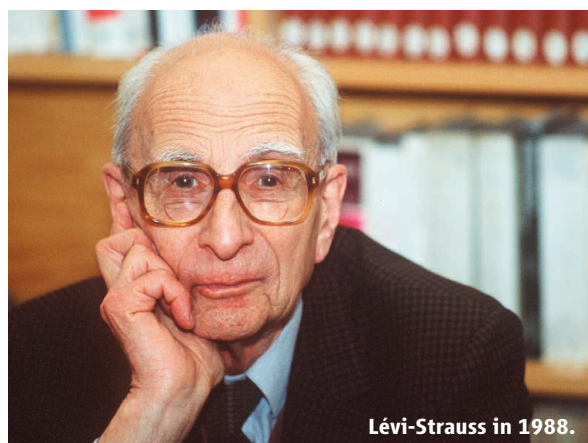
The Structuralist Savant

Paul Shankman

When French anthropologist Claude Lévi-Strauss died last year at the age of 100, the world was reminded once again of his importance as the founder and major proponent of structuralism, a school of thought that had considerable currency in European and American intellectual circles from the 1950s through the 1970s. Patrick Wilcken's well-written biography, the first full-length treatment of Lévi-Strauss's life in English, provides an accessible and interesting overview of his career, personal life, intellectual development, contributions, and impact on thinking inside and outside of anthropology. Wilcken offers a clear explication and measured assessments of Lévi-Strauss's works as well as setting them within the broader context of 20th-century French and European thought.

Lévi-Strauss initially conceived of structuralism as a way of making the humanities more scientific, grounding his work in a blend of classical European philosophy and modernist thinking. As a young man, he was "an intellectual omnivore, a grazer eternally on the move, roaming the vast plains of Western culture—moving from French literature to philosophy to modernism across the arts." In the late 1930s, after earning degrees in philosophy and law, he changed direction, launching an ethnographic expedition into the Brazilian interior to study some of the remaining tribal societies and to collect artifacts from them. After this rather limited and often difficult experience, recounted in Lévi-Strauss's well-known *Tristes Tropiques* (1), he would never conduct field research again.

On returning to France, Lévi-Strauss sought ways of moving beyond the narrow confines of ethnographic description to the study of universal laws about the workings of the human mind. Unfortunately, World War II interrupted his professional development. As a Jewish refugee, he fled to New York City,



Lévi-Strauss in 1988.

where he embraced the work of Russian linguist and fellow exile Roman Jakobson. A merger of linguistics and anthropology would become the basis of structural analysis, with Lévi-Strauss nurturing the idea that cultural phenomena, such as myth, kinship, art, and cooking, were like language—each with surface meanings underpinned by deeper structures. Thus, Lévi-Strauss could break down a myth into its constituent elements and then examine the general relationships among elements, allowing him to transform the chaos of ethnographic detail into the order of structural regularities as he perceived them.

After the war, Lévi-Strauss returned to France, where he received his Ph.D. in anthropology on the eve of his 40th birthday. He then gradually rose to the highest levels of the French academy. By the mid-1960s, there was a growing intellectual movement that found Lévi-Strauss at its center in the company of other prominent social thinkers, including Louis Althusser, Roland Barthes, Michel Foucault, and Jacques Lacan. They would shape a generation of social thought for much of Europe. With translations

of his books and articles appearing in English, Lévi-Strauss was now widely acclaimed and sought after, achieving celebrity status across the globe. The breadth of his scholarship was on display in a series of books ranging from *The Elementary Structures of Kinship* (2) through *The Savage Mind* (3) to his monumental four-volume *Mythologiques* (4–7), in which he analyzed indigenous myths throughout the Americas.

Yet as Lévi-Strauss's reputation was approaching its zenith, structuralism itself was falling out of fashion. The central problem was structuralism's dependence on the idiosyncratic insight of its individual prac-

tioners. Structural analyses were difficult if not impossible to replicate and compromised by their possible distortion of the ethnographic record and their neglect of developments in cognitive science. Reviewing structuralism's limitations, Wilcken finds it "a strange kind of science" and notes the "curious paradox in all Lévi-Strauss's writing that at the very moment he evokes science in his defense, a kind of mysticism is not far behind."

Lévi-Strauss was, in Wilcken's view, a "poet in the laboratory" and his work a kind of "Zen anthropology," noble in its pursuits but "quixotic" in its methods. It is therefore not surprising that by the 1970s interest in structuralism began to wane. By the 1990s, Lévi-Strauss was virtually the last remaining structuralist, continuing to produce additional works in that genre until his death. Although Lévi-Strauss recognized the problems that structuralism posed and attempted to distance himself from his creation, he saw no alternative to it.

Wilcken's biography is a reliable guide to Lévi-Strauss's life and work. He interviewed Lévi-Strauss during his last years, and the book benefits from these interviews and personal anecdotes. (No, Lévi-Strauss was not related to the famous maker of jeans.) Wilcken is also a gifted storyteller and thoughtful analyst. Some readers may wish for more discussion of Lévi-Strauss's successors (the post-structuralists) or additional material on Lévi-Strauss's influence on American anthropology, but there are and there will be other works evaluating this great figure's legacy. For Wilcken, what is most noteworthy about Lévi-Strauss is his influence far beyond anthropology and into such diverse fields as literature, philosophy, art, history, politics, and linguistics. He concludes that, despite structuralism's limitations, "In a world of ever more specialized areas of knowledge, there may never again be a body of work of such exhilarating reach and ambition."

References

1. C. Lévi-Strauss, *Tristes Tropiques* (Plon, Paris, 1955).
2. C. Lévi-Strauss, *Les structures élémentaires de la parenté* (Presses universitaires de France, Paris, 1949).
3. C. Lévi-Strauss, *La pensée sauvage* (Plon, Paris, 1962).
4. C. Lévi-Strauss, *Le Cru et le cuit* (Plon, Paris, 1964).
5. C. Lévi-Strauss, *Du miel aux cendres* (Plon, Paris, 1966).
6. C. Lévi-Strauss, *L'Origine des manières de table* (Plon, Paris, 1968).
7. C. Lévi-Strauss, *L'homme nu* (Plon, Paris, 1971).

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ENVIRONMENT AND DEVELOPMENT

The Energy-Poverty-Climate Nexus

Christian E. Casillas^{1,3} and Daniel M. Kammen^{1,2,3,4*}

Close to two-thirds of the world's poorest people live in rural areas (1). Eradication of rural poverty depends on increased access to goods, services, and information, targets detailed in the United Nations Millennium Development Goals. However, alleviating poverty is hindered by two interlinked phenomena: lack of access to improved energy services and worsening environmental shocks due to climate change. Mitigating climate change, increasing energy access, and alleviating rural poverty can all be complementary, their overlap defining an energy-poverty-climate nexus. We describe interventions in a rural Nicaraguan community to show that energy services can be provided in cost-effective manners, offering the potential to address aspects of rural poverty while also transitioning away from fossil fuel dependence.

The Energy-Poverty-Climate Nexus

Increased access to energy services alone will not eradicate poverty, but it can have immediate effects (2, 3). More than 1.5 billion people live without access to electricity, another billion only have access to unreliable electricity, and close to half the global population depends on traditional biomass fuels for cooking and heating (4). Energy poverty results in unmet basic needs and depressed economic and educational opportunities that are particularly pervasive among women, children, and minorities (5, 6). Electricity catalyzes rural economic activity (7–10) and increases the quality of services available to meet basic business and domestic needs through improved lighting, labor-saving devices, and access to information through TV, radio, and cellular telephones (11). Provision of high-quality public lighting can increase security and improve delivery of health and education services (7, 11).

Environmental shocks related to climate change will first and most severely affect

vulnerable, poor populations, many living in rural areas (1, 12). Improving delivery of affordable, reliable energy services to rural communities is critical for helping them develop human and economic capacity to adapt in the face of a changing climate.

Greenhouse gas emissions in industrial-

"Mitigating climate change, increasing energy access, and alleviating rural poverty can all be complementary."

ized countries are dominated by electricity generation and transportation, whereas the majority of emissions from the world's poorest countries come from agriculture and changes in land use (1). However, with 1.5 billion people without access to electricity, combustion-related emissions from the rural power sector are expected to grow. Because of low capital costs and a large network of suppliers, diesel generators are often the technology of choice in rural areas, without sufficient consideration of the volatility of fuel prices, resulting in expensive generation costs (9, 13).

Given the relationships outlined above, every dollar spent on the transition to more

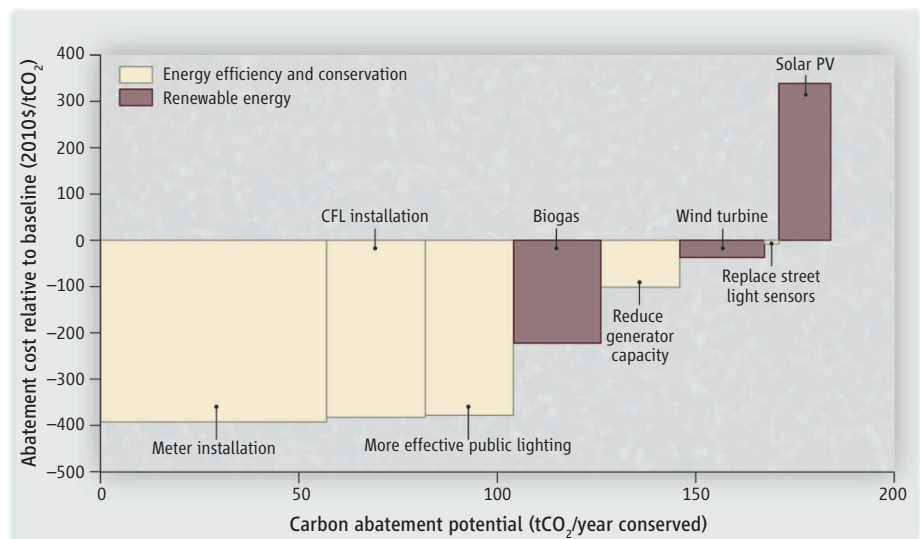
Community-level carbon abatement curves highlight opportunities for increased access to clean, efficient energy for the poor.

efficient low-carbon energy systems in rural areas has the potential to produce greater human development, savings, and carbon mitigation returns than in more industrialized areas (if economies of scale do not dominate). However, debates about climate change and vulnerability have been slow to highlight the energy-poverty-climate nexus. This has been due, in part, to the lack of meaningful metrics needed to stimulate social, economic, and technical innovation in this sector.

Marginal Abatement Cost Curves

A marginal abatement cost (MAC) curve typically shows the annual carbon abatement potential for an intervention, and the cost per quantity of carbon emissions abated, relative to the emission costs for a baseline case (14, 15). A community-level MAC curve derived from ongoing research on the Atlantic coast of Nicaragua demonstrates that low-carbon rural energy services can be delivered at cost savings in cases where communities use diesel-powered generation, isolated from the national grid (microgrids).

The rural communities of Orinoco and Marshall Point share a diesel microgrid serving 172 households. In partnership with the Nicaraguan government and a local nongov-



MAC curve of the electricity sector for Orinoco and Marshall Point. Abatement cost is with respect to a baseline diesel carbon price of \$397 per metric ton of CO₂ (tCO₂). (Negative cost indicates savings.) Abatement potential is due to the reduction of diesel use, relative to each previous measure. Multiplying abatement potential and abatement cost gives total annual costs relative to baseline, assuming that the previous measure was implemented. Only the most economic technologies appropriate for the community resources, capacity, and grid sophistication are included. See SOM for details.

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ernment organization, several energy efficiency measures were implemented in 2009 [see the supporting online material (SOM)]. Based on this work, we developed a MAC curve for the electricity sector of these communities (see the figure). The first two efficiency measures in the curve [installation of meters and compact fluorescent lights (CFLs)] were actually implemented, whereas impacts of subsequent measures are based on estimations (SOM).

With the price of diesel fuel at US\$1.06 per liter, the generation cost for each additional unit of electricity in the village (its marginal generation cost) is \$0.54 per kilowatt-hour (kWh) (SOM), compared with costs on the order of \$0.10 per kWh in the national grid (16). This difference in generation costs creates the potential for greater savings available from mitigation in diesel microgrids (although the total capacity for carbon abatement is considerably less than in the national grid). The majority of the abatement measures in the figure can be achieved at negative costs relative to the diesel baseline (i.e., costs are outweighed by savings).

There are a number of ways an intervention's impact on poverty can be assessed. For this study, we quantify the potential for increase in availability of energy and reduction in household consumption, which can translate to reduced expenditures without decreasing the quality of energy service. Future work could explore how interventions create jobs and increase earnings and how benefits are distributed by using inequality metrics such as the Gini coefficient or Kuznets ratios.

Decreasing Consumption

The installation of electricity meters allowed accurate billing of household consumption, instead of using unmetered, fixed tariffs. This resulted in a 28% decrease in daily energy consumption, which could translate into household savings. The relatively greater reduction in daytime load suggests that meter installation resulted in reduction of less-valued energy services (e.g., lights being left on during the day).

To increase lighting efficiency, every household was given the option to replace two incandescent bulbs with CFLs, resulting in an additional 17% drop in daily consumption and the potential for additional household savings. The large demand response due to metering and efficient lighting was the result of both behavioral changes and a market intervention (17, 18).

The combination of the meter and CFL installations led to an increased availabil-

ity of 84 liters of diesel per day. The daily operation of the microgrid was increased by 2 hours, providing households the opportunity to invest in additional electricity use (19). In the month following the two measures, 37% of the households in Orinoco received lower electricity bills. However, benefits to the poorest households were mitigated due to a regressive tariff structure in which the smallest consumers pay a fixed rate (SOM).

The MAC curve also highlights estimated benefits of replacing a portion of diesel fuel with biogas. The biogas can be produced locally through anaerobic digestion of animal dung and agricultural residues. This introduces the opportunity for a large part of the gross carbon abatement cost to be captured within the community through local, low-carbon fuel production, rather than paying for imported fossil fuel. Although community-scale biogas systems have had mixed success, often depending on the model of ownership, they highlight opportunities for implementing sustainable biofuel systems with current technology (20).

Suite of Tools for Poverty-Climate Analysis

Although this MAC curve focused on carbon abatement in the electricity sector, similar curves can be created for different rural energy services such as cooking and transportation, as well as agriculture. For example, 57% of households use charcoal for cooking, with the majority having unimproved stoves. More efficient stoves would mitigate black carbon emissions, lessening impacts on climate and also respiratory harm most prominent among women and children (21, 22).

Using MAC curves in conjunction with a clear understanding of how various measures will support community development goals ensures that climate change dollars also address the most pressing challenges of the poorest communities. However, MAC curves must be part of a suite of analytic tools for understanding various poverty-climate nexuses. For example, investment in agro-ecological farming practices may not necessarily appear favorable in a MAC curve but will likely be critical in the agricultural-poverty-climate nexus (23).

Integration of development agendas into climate change frameworks has been limited, in part, by a lack of both easy-to-understand metrics and systems-level planning tools necessary for prioritizing the allocation of limited capital. Using one such tool, MAC curves, it is apparent that increasing access to energy services can reduce carbon emissions and monetary expenditures, with great potential to affect development and reduce poverty.

Continued development of methods of analysis, and interventions based on those analyses, is needed to allow us to reduce poverty while also confronting climate change.

References and Notes

1. R. Bierbaum, M. Fay, *World Development Report 2010: Development and Climate Change* (World Bank, Washington, DC, 2010).
2. V. Modi, S. McDade, D. Lallement, J. Saghir, *Energy Services for the Millennium Development Goals* (World Bank, New York, 2005).
3. E. Mills, *Science* **308**, 1263 (2005).
4. United Nations Development Programme, *Energy for a Sustainable Future: The Secretary-General's Advisory Group on Energy and Climate Change Summary Report and Recommendations* (UNDP, New York, 2010).
5. International Energy Agency, *World Energy Outlook* (IEA, Paris, 2005).
6. A. Jacobson, A. D. Milman, D. M. Kammen, *Energy Policy* **33**, 1825 (2005).
7. E. Cecelski, *Enabling Equitable Access to Rural Electrification: Current Thinking and Major Activities in Energy, Poverty and Gender* (World Bank, Washington, DC, 2000).
8. R. A. Cabraal, D. F. Barnes, S. G. Agarwal, *Annu. Rev. Environ. Resour.* **30**, 117 (2005).
9. C. Flavin, M. H. Aeck, *Energy for Development: The Potential Role of Renewable Energy in Meeting the Millennium Development Goals* (Worldwatch Institute, Washington, DC, 2005).
10. C. Kirubi, A. Jacobson, D. M. Kammen, A. Mills, *World Dev.* **37**, 1208 (2009).
11. Independent Evaluation Group, *The Welfare Impact of Rural Electrification: A Reassessment of the Costs and Benefits* (World Bank, Washington, DC, 2008).
12. Intergovernmental Panel on Climate Change, *Climate Change 2007: Impacts, Adaptation and Vulnerability* (Cambridge Univ. Press, Cambridge, 2007).
13. Energy Sector Management Assistance Programme, *Technical and Economic Assessment of Off-Grid, Mini-Grid and Grid Electrification Technologies* (World Bank, Washington, DC, 2007).
14. McKinsey & Company, *Pathways to a Low-Carbon Economy* (McKinsey, London, 2009).
15. T. M. Johnson, C. Alatorre, Z. Romo, F. Liu, *Low-Carbon Development for Mexico* (World Bank Publications, Washington, DC, 2009).
16. W. Mostert, *Unlocking Potential, Reducing Risk: Renewable Energy Policies for Nicaragua* (World Bank, Washington, DC, 2007).
17. A. B. Jaffe, R. N. Stavins, *Energy Policy* **22**, 804 (1994).
18. P. C. Stern, G. T. Gardner, M. P. Vandenbergh, T. Dietz, J. M. Gilligan, *Environ. Sci. Technol.* **44**, 4847 (2010).
19. In the case where an electricity grid is operating for only a fixed number of hours each day, based on a limited supply of fuel, increased demand-side efficiency allows for fuel savings to be invested in longer hours of operation, which will not immediately result in a reduction of total carbon emissions but rather a reduction of carbon emissions per delivery of energy services.
20. H. Romijn, R. Raven, I. de Visser, *Environ. Sci. Policy* **13**, 326 (2010).
21. K. R. Smith *et al.*, *J. Expo. Sci. Environ. Epidemiol.* **20**, 406 (2010).
22. M. Ezzati, D. M. Kammen, *Lancet* **358**, 619 (2001).
23. M. A. Altieri, *Agric. Ecosyst. Environ.* **93**, 1 (2002).
24. We thank blueEnergy, and its directors M. and G. Craig, for resources and support and R. Ghanadan, A. Kantanbacher, A. Mason, I. Ray, and reviewers for comments. C.E.C. is an advisor to blueEnergy. This work was supported by the Energy Foundation, the Karsten Family Foundation, and the Class of 1935 of the University of California, Berkeley.

Supporting Online Material

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CELL BIOLOGY

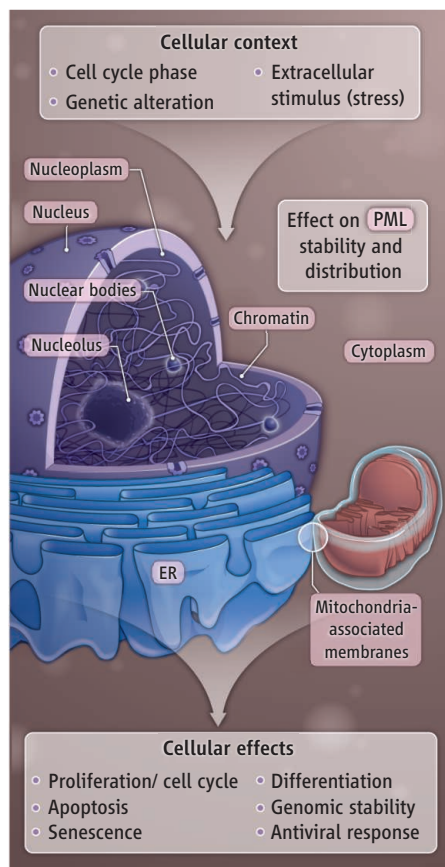
Puzzled by PML

Bijana Culjkovic-Kraljic and Katherine L. B. Borden

Promyelocytic leukemia (PML) protein nuclear bodies are one of many structures found in the nucleus of higher eukaryotes. There are about 10 to 30 of these spherical bodies per nucleus, ranging in size from ~0.2 to 1 μm in diameter. PML nuclear bodies (also known as PML oncogenic domains, Kremer bodies, or nuclear dot 10s) are currently defined by the presence of the PML protein (1). They were first identified in the 1960s, but their relationship with PML protein was only described about 20 years ago (2, 3). PML protein inhibits cell cycle progression, promotes cell death, suppresses some types of oncogenic transformation and senescence, acts in stem cell renewal, and plays a role in defense against many viruses (1–5). Although these effects are well described, the underlying molecular mechanisms are not. On page 1247 of this issue, Giorgi *et al.* (6) propose that PML protein controls calcium signaling at the endoplasmic reticulum (ER), potentially increasing its functional diversity.

How does PML protein act in the diverse processes of DNA repair, DNA replication, transcription, repression of messenger RNA (mRNA) nuclear export, and posttranslational modifications of proteins (1–5)? To date, only two discrete biochemical activities of PML protein have been demonstrated: It acts as an E3 ligase that interacts with the enzyme Ubc9 to attach the protein SUMO to target proteins (7); and it binds to and alters the conformation of eukaryotic translation initiation factor 4E (eIF4E), thereby impairing eIF4E-dependent mRNA nuclear export (4, 8). Generally, disruption of PML nuclear bodies reflects a loss of its biochemical activities and physiological effects. No unifying principle for the physiological effects of PML protein has been discovered, which may be due, in part, to the heterogeneity and dynamic nature of the PML nuclear bodies themselves.

Another complication is that not all PML protein is found in nuclear bodies. A substantial fraction is found in the nucleoplasm, and some attribute its functionality to this fraction (2). PML protein also accumulates in the nucleolus in response to cellular stress and senescence (3, 5). Although a small fraction of PML protein is in the cytoplasm under nor-



PML in the cell. Expression of PML protein isoforms and their subcellular distribution depends on cellular context and has different effects on cell fate.

mal conditions, it can become enriched in the cytoplasm in specific pathogenic conditions (1, 4, 5). The human *PML* gene can be alternatively spliced, and there are multiple isoforms (3, 5). Some isoforms do not contain a nuclear localization signal; PML I (the longest isoform) contains both a nuclear localization and a nuclear export signal (1, 3, 5). PML protein can also be posttranslationally modified, potentially altering its location (9).

In the cytoplasm, a fraction of PML protein binds to eIF4E, and in vitro, PML protein can repress translation (1, 4). During mitosis, PML protein distributes to mitotic accumulations of PML protein (MAPPs) (10). A mutant form of PML protein (cPML) that is predominantly cytoplasmic appears to sequester coactivators of the tumor suppressor protein p53, thereby inhibiting p53 function (5). cPML may control the effects of transforming growth factor- β (TGF- β)

A tumor suppressor protein may regulate cell survival by controlling the release of calcium from intracellular stores.

by interacting with SMADs, the intracellular proteins that transduce signals from this factor (11). Thus, the variety of PML protein isoforms, coupled with posttranslational modifications, likely underlies the diversity in PML functions, perhaps acting in distinct biochemistries in different cellular contexts.

Giorgi *et al.* provide evidence that in mouse fibroblasts, some PML protein is found at the ER and near mitochondria. Their findings suggest that loss of PML protein is associated with reduced calcium release from the ER, leading to reduced apoptosis in response to ER-dependent stresses. The authors propose that PML protein modulates phosphorylation of the inositol 1,4,5-trisphosphate receptor (IP₃R) and subsequent IP₃R-mediated calcium release from ER. This pathway could depend on the protein kinase Akt and protein phosphatase PP2A. The finding is consistent with proposed apoptotic functions of PML protein that can be independent of its roles in transcription (4, 12).

Given that there is no reported ER (or mitochondrial) targeting sequence in PML protein, what regulates its trafficking to the ER? Perhaps only a subset of PML variants or specific modified forms localize to the ER. How does the PML protein found at the ER, which constitutes a small fraction of the total PML protein (6), have such a disproportionate impact on cell physiology? Some fraction of PML protein may also modulate calcium signaling from the recently identified nucleoplasmic reticulum (13). The larger question is how the many disparate PML protein activities are connected and coordinated.

The findings of Giorgi *et al.* may serve as a focal point for the emergence of unifying biochemical principles for PML protein activities. PML protein function may differ temporally and depend on cellular context, thereby providing plasticity and specificity (see the figure). Determining the functions of PML protein has traditionally been based on the activities of its protein partners. Although helpful, this strategy can be unreliable because the relevant functions of binding partners may not yet be known. This complicates building a unifying model for PML protein functions. More in-depth analyses of PML protein biochemistry both within and outside nuclear bodies will be critical. Clearly, we are still puzzled by the PML protein after all these years.

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References and Notes

1. K. L. Borden, *Mol. Cell. Biol.* **22**, 5259 (2002).
2. G. G. Maul *et al.*, *J. Struct. Biol.* **129**, 278 (2000).
3. V. Lallemand-Breitenbach, H. de Thé, *Cold Spring Harb. Perspect. Biol.* **2**, a000661 (2010).
4. K. L. Borden, B. Culjkovic, *Front. Biosci.* **14**, 497 (2009).
5. P. Salomoni *et al.*, *Cell Res.* **18**, 622 (2008).
6. C. Giorgi *et al.*, *Science* **330**, 1247 (2010); 10.1126/science.1189157.
7. E. Duprez *et al.*, *J. Cell Sci.* **112**, 381 (1999).
8. L. Volpon *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **107**, 5441 (2010).
9. J. N. Nichol *et al.*, *Front. Biosci.* **14**, 2293 (2009).
10. G. Dellaire *et al.*, *J. Cell Sci.* **119**, 1034 (2006).
11. H. K. Lin *et al.*, *Nature* **431**, 205 (2004).
12. F. Quignon *et al.*, *Nat. Genet.* **20**, 259 (1998).
13. W. Echevarría *et al.*, *Nat. Cell Biol.* **5**, 440 (2003).
14. K.L.B.B. holds a Canada Research Chair and is supported by the National Institutes of Health and the Leukemia and Lymphoma Society. The Institute of Research in Immunology and Cancer receives infrastructure support from the Fonds de la Recherche en Sante du Quebec and Canadian Institutes of Health Research.

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ASTRONOMY

Cosmic Rays from Cosmic Birth

Tom Ray

Jets of plasma containing energized charged particles have been observed emanating from a host of astrophysical objects, including massive black holes at the center of active galaxies, gamma-ray bursters, planetary nebulae, young stars, and brown dwarfs. There are good reasons for suspecting that the same basic mechanism generates the jet in every case despite the mass differences spanning more than 10 orders of magnitude (1). All of these objects have several features in common: a central gravitating source, an accretion disk (which is formed from diffuse matter orbiting around a central mass), and a strong magnetic field. On page 1209 of this issue, Carrasco-González *et al.* (2) show that these objects also share another characteristic—the ability to accelerate electrons to highly relativistic energies. The observations provide a key step in our understanding of the mechanisms of jet formation.

Various theories have been put forward as to how jets form, but only one has stood the test of time, i.e., a jet starts as a fan-shaped wind that is ejected from the accretion disk and flung outwards on magnetic field lines (3, 4). As the material moves away from the disk, it is forced to follow the field, rather like the solar wind particles that create aurorae on Earth. However, because the field is anchored to the spinning accretion disk, it also rotates, and thus the velocity of the jet material increases as it moves outward. Eventually the ejected material moves so fast that it bends back the magnetic field lines and the field becomes doughnut-shaped rather than fan-shaped. The field lines then start to focus the material, and a jet is born.

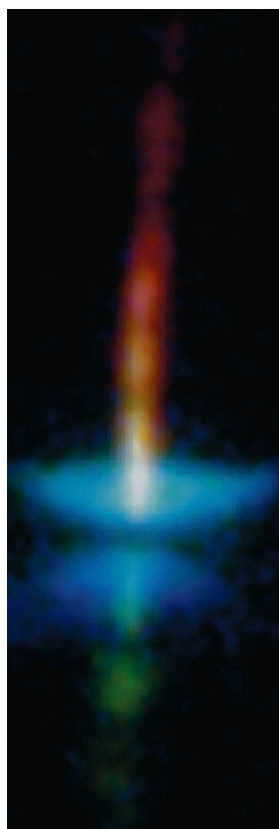
Although this picture is generally accepted theoretically, there is little supporting, observational evidence, primarily because the physical data on individual jets are incomplete. For example, jets from active galactic nuclei have been mapped in exquisite detail by radio interferometers such as the Very Large Array (VLA) in New Mexico (5). These jets radiate through synchrotron emission, the energy emitted in the form of radio waves by relativistic electrons as they spiral around magnetic fields. Although the intensity and polarization of the emitted synchrotron radiation can be used to estimate the strength and direction, respectively, of the magnetic field (6), relatively little is known about the other basic parameters such as the velocity of the

jet and its mass loss rate. In comparison, an enormous amount of detail is known about jets from young stars. This is not only because they are relatively nearby and thus can be explored with high spatial resolution (7) (see the figure), but also because they primarily produce narrow emission lines unlike the broad continuum emitted by jets from active galaxies. Fundamental parameters such as the density, temperature, and ionization fraction in a jet can be determined from the

Observations of radio emission from young stellar objects provide key information to help us unravel the formation mechanism of astrophysical jets.

ratio of various emission lines (8). Moreover, by gauging the shift in the lines as a result of the Doppler effect, a jet's radial velocity can be measured, and combining this information with images taken a few years apart, the full three-dimensional velocity of the jet can be ascertained. The one missing ingredient for obtaining a complete physical census of jets from young stars is the strength of their magnetic field, ironically perhaps the only parameter that is well determined in the case of their larger brethren. Carrasco-González *et al.* show that jets from young stars emit synchrotron radiation in addition to the known plethora of emission lines. This allows us to measure the strength and direction of their magnetic fields directly.

At first glance, the discovery of synchrotron emission from jets from young stars is surprising. The typical temperature in a jet from a young star is only a few thousand degrees, so any electrons present should not have relativistic energies. But this ignores an important fact about their radiation: that it comes from the cooling zone behind a shock. Particles like electrons and protons gain energy simply by diffusing upstream across a shock front (9). The shock then catches up with the particle, but it diffuses across it again, gaining even more energy as it does so. This process, known as diffusive shock acceleration, can cause an electron moving at a few hundreds of kilometers per second to reach velocities near the speed of light. It is also thought to be the mechanism that gives rise to cosmic rays (10). Diffusive shock acceleration seems to work in jets from active galaxies and supernova remnants, but in those cases the shocks have velocities of at least several thousand kilometers per second.



Young, but powerful. Jets from a young star can produce relativistic particles, as shown by Carrasco-González *et al.* This high-resolution Hubble Space Telescope image shows the bipolar jet (red and green) from the young star known as Herbig-Haro 30. Its accretion disk, comparable in size to the solar system, is seen in silhouette, and light from the star is scattered (blue) above and below the disk (7).

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The shocks in jets from young stars are much slower, at most a few hundred kilometers per second, but the new findings suggest that diffusive shock acceleration can operate even in these puny jets. Thus, jets from young stars could be a new extreme testing ground for the theory of how cosmic rays are made.

Looking ahead, radio astronomy is currently undergoing a revolution. Fiber-optic cables have replaced microwave links, enabling much larger portions of the spectrum to be received and analyzed by radio interferometers. This revolution is akin to the introduction of digital cameras in optical astronomy three decades ago when the efficiency of a telescope at collecting photons was increased from a few percent with photographic plates, to more than 70%. Refurbished radio interferometers, such as the Expanded VLA (EVLA) in the United States (11) and the extended-Multi-Element Radio Linked Interferometer Network (e-MER-

LIN) in the United Kingdom (12), will be at least an order of magnitude more sensitive than their predecessors. This will have a major impact on many areas of astronomy, including star formation. Observing jets from young stars is just at the limit of what existing radio telescopes can usefully do. Although most of their radio emission is thermal due to ordinary electrons being released by collisions in the gas, the increased sensitivity of the new radio telescopes should enable the discovery of many more nonthermal (synchrotron) jets from young stars and nonthermal components in previously known, predominantly thermal, jets.

The observations reported by Carrasco-González *et al.* finally provide a means of determining the structure and strength of the last poorly known parameter in stellar jets, i.e., the magnetic field, and thus provide a crucial test of the jet-formation paradigm. Ironically while the vast majority of cosmic rays

may be generated when stars die, a very small fraction could be produced at their birth.

References

1. M. Livio, *Phys. Rep.* **311**, 225 (1999).
2. C. Carrasco-González *et al.*, *Science* **330**, 1209 (2010).
3. R. E. Pudritz, R. Ouyed, C. Fendt, A. Brandenburg, in *Protostars and Planets*, V. B. Reipurth, D. Jewitt, K. Keil, Eds. (Univ. of Arizona Press, Tucson, 2007), pp. 277–294.
4. F. H. Shu, J. R. Najita, H. Shang, Z.-Y. Li, in *Protostars and Planets IV*, V. Mannings, A. P. Boss, S. S. Russell, Eds. (Univ. of Arizona Press, Tucson, 2000), pp. 789–813.
5. W. B. Sparks *et al.*, *Astrophys. J.* **473**, 254 (1996).
6. F. Dulwich, D. M. Worrall, M. Birkinshaw, C. A. Padgett, E. S. Perlman, *Mon. Not. R. Astron. Soc.* **374**, 1216 (2007).
7. T. P. Ray *et al.*, *Astrophys. J.* **468**, L103 (1996).
8. T. Ray *et al.*, in *Protostars and Planets*, V. B. Reipurth, D. Jewitt, K. Keil, Eds. (Univ. of Arizona Press, Tucson, 2007), pp. 231–244.
9. L. O. C. Drury, *Mon. Not. R. Astron. Soc.* **251**, 340 (1991).
10. S. P. Reynolds, *Annu. Rev. Astron. Astrophys.* **46**, 89 (2008).
11. R. Perley *et al.*, *IEEE Proc.* **97**, 1448 (2009).
12. S. T. Garrington *et al.*, in *Society of Photo-Optical Instrumentation Engineers (SPIE) Conference Series*. (2004), vol. 5489, pp. 332–343.

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CELL BIOLOGY

The Case for RNA

Chang C. Liu^{1,2} and Adam P. Arkin^{1,3,4}

A major challenge in cell-based biotechnology is to engineer gene regulatory systems that can detect signals defined by users and then effect desired changes in the expression of targeted genes. On page 1251 of this issue, Culler *et al.* (1) take an innovative step toward meeting this challenge. They describe the use of short segments of RNA to create programmable “control devices” that, when triggered by the presence of certain proteins, rewire gene expression pathways and change the behavior of human cells. The work highlights the promise of RNA as a molecular platform for engineering predictable and customized gene regulation systems and potentially using such synthetic systems to treat disease.

Cells are masters of regulating genes in response to environmental cues. They accomplish this through an overwhelming variety of mechanisms, including riboswitches, RNA silencing, protein transcription factors, post-translational modification, and degradation. This mechanistic diversity is represented

in differences in the speed, dynamic range, cooperativity, and functional complexity of gene regulation. Bioengineers, however, have had difficulty appropriating such diversity because each mechanism demands a distinct set of strategies in order to tailor it for synthetic systems. From an engineering perspective, it would be more useful to create a simple yet complete framework, ideally based on one molecular system, for building synthetic gene regulation systems. Is such a framework possible?

In the past 20 years, RNA biotechnologists have aimed to answer “yes.” They have pursued a general strategy of coupling RNA aptamers (RNA structures that recognize, or bind to, specific small molecules or proteins) to the transcription, translation, or processing of genes (2–5). They have demonstrated that this approach can produce “user-defined” gene expression control systems (6–8).

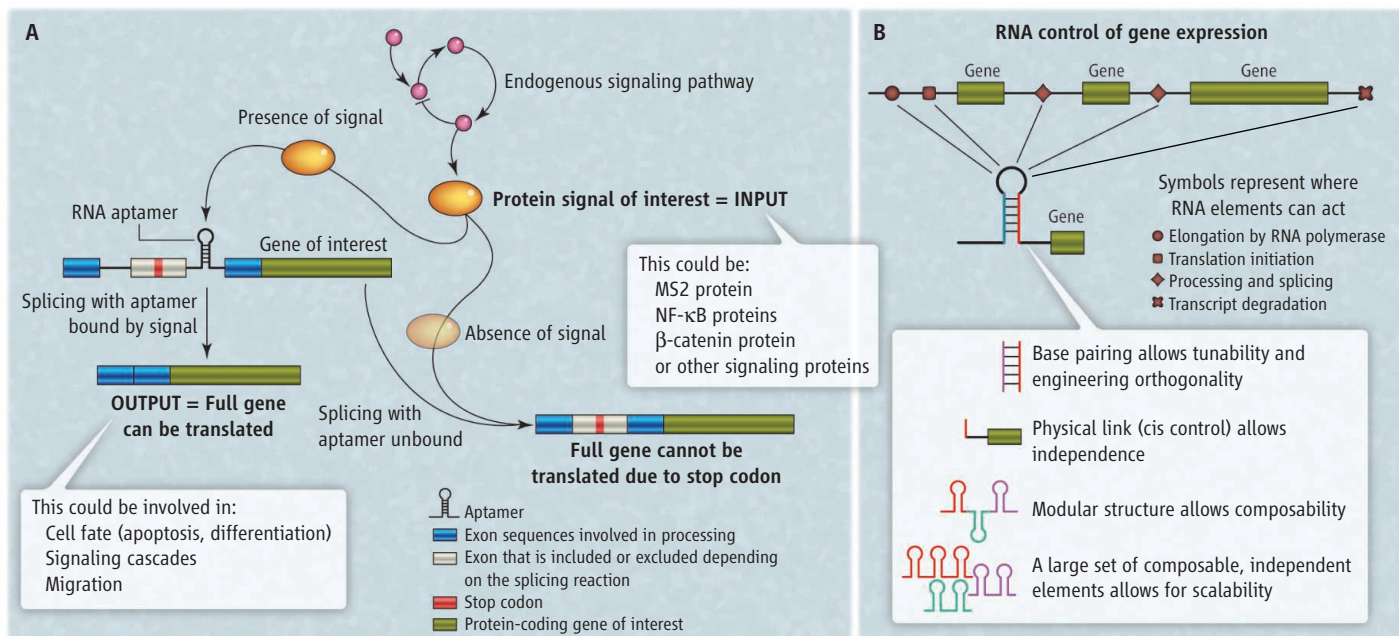
Culler *et al.* present an inventive instantiation of this strategy. Expanding on previous work in engineered alternative splicing systems (9, 10), they place aptamers that bind specific proteins into introns (eukaryotic DNA regions within a gene that are not translated into proteins) and are able to show that the DNA is spliced differently, depending on whether the aptamer is bound to its cognate (matching) protein “signal.” When they

RNA shows promise for engineering synthetic systems for controlling gene expression.

add a stop codon in an exon that, because of the differential splicing, is either included or excluded in the final messenger RNA (mRNA), a direct change in the translation of the processed gene results (see the figure). This procedure constitutes a versatile method for engineering user-defined gene regulation; in theory, any intracellular protein can be made to trigger an increase or decrease in the expression of any fused gene.

Culler *et al.* demonstrate the function of these systems in human cells with the use of introns containing aptamers for the proteins MS2, β -catenin, or nuclear factor κ B (NF- κ B), and present an example of combinatorial regulation in which the RNA devices sense two protein inputs. Then, they show that their devices can be used to synthetically rewire cellular behavior. In their proof-of-principle experiment, they link stimulation of the Wnt or NF- κ B signaling pathways to the expression of a gene that confers sensitivity to the drug ganciclovir, which induces programmed cell death (apoptosis). This experiment demonstrates the modularity of their strategy and shows that endogenous cellular signaling pathways can be rerouted to guide cells to particular fates. This is an important goal for cell biology and cell-based therapy. It even raises the intriguing possibility of killing diseased cells by using gene therapy

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Engineering RNA controls. (A) Placing an RNA aptamer sensitive to a specific protein signal (orange) into an intron causes the transcript to splice differently in response to the signal. Adding a stop codon (red) within an exon (white) turns this splicing difference into a difference in the translation of the targeted gene (green). In this case, aptamer bound to signal allows full gene to be translated

(left), but unbound aptamer prevents complete translation (right). (B) RNA elements exhibit a number of properties useful for engineering gene controls—including independence, tunability, orthogonality, composability, and scalability—and are involved in a number of key processes, including elongation, translation initiation, splicing, and degradation.

techniques to synthetically connect disease-associated pathways to apoptosis.

The strategy is not perfect. Because bioengineers do not completely understand the mechanisms underlying the system, combinatorial regulation does not yet produce predictable effects, and gene expression changes in response to the input signals are moderate (a factor of 2 to 4 in the best cases). New developments in analyzing RNA structures, however, should help researchers to overcome these difficulties and tap the great potential this strategy holds for rewiring cellular function.

As for the larger goal of creating a complete framework for the predictable design of gene regulation systems, the choice to engineer RNA is a good one. RNA elements—whether aptamer-based systems, synthetic riboswitches, antisense-mediated regulation, or combinations of these (11)—can satisfy the properties required for a complete gene regulation engineering platform. These properties, as defined by Lucks *et al.* (12), include independence, tunability, orthogonality, scalability, and composability. For example, RNA elements usually act in cis (on the same molecule), so their effect is physically constrained to the regulated gene(s). This feature gives RNA gene-regulatory elements a good chance of being independent of each other and of other cellular processes. RNA also folds into secondary structures predictably, according to base-pairing rules; hence, it is

feasible to engineer variant or new structures and mechanisms either de novo or by manipulating RNA elements naturally involved in gene regulation. RNA parts should therefore be tunable for function at the desired levels. In addition, RNA's structural reliance on predictable base pairing gives bioengineers the ability to take a parent RNA part and systematically create sets of orthogonal variants, meaning that these variants function in a similar manner but do not “cross talk” or interact with each other. These sets can be designed to respond to different inputs, thus turning one RNA part into a family of interoperable parts.

RNA elements are also highly scalable. Powerful in vitro selection techniques can yield custom aptamers that bind to user-defined signals or ribozymes that perform user-defined functions (13). These selection techniques, combined with the fact that natural RNA elements are involved in all steps of gene regulation, translate into a huge number of detectable signals and a very wide variety of gene regulation output types that can be engineered. This scalable diversity has the potential to satisfy the demands of any desired synthetic gene-regulatory system. In addition, RNA folds into modular domains, which means that individual RNA parts can be assembled together to achieve combinatorial regulation and higher-order function. RNA parts are therefore composable, and if bioengineers are able to establish general conventions for connecting RNA elements

together, they could quickly craft new regulatory units with complex but predictable functions. Finally, the rules for RNA folding are similar across different organisms, increasing the likelihood that systems engineered in one organism will be portable to another (7).

Together, these properties suggest that RNA elements may soon constitute a general and highly applicable platform for engineering gene regulation systems that satisfy the needs of cell-based biotechnology (14, 15). Even if individual efforts fall short of this goal, bioengineers should be able to synthesize varying strategies into a unified framework that is more than the sum of its (RNA) parts.

References

1. S. J. Culler *et al.*, *Science* **330**, 1251 (2010).
2. K. H. Link, R. R. Breaker, *Gene Ther.* **16**, 1189 (2009).
3. S. Topp, J. P. Gallivan, *ACS Chem. Biol.* **5**, 139 (2010).
4. A. D. Ellington, *ACS Chem. Biol.* **2**, 445 (2007).
5. M. N. Win *et al.*, *Chem. Biol.* **16**, 298 (2009).
6. G. Werstuck, M. R. Green, *Science* **282**, 296 (1998).
7. M. N. Win, C. D. Smolke, *Proc. Natl. Acad. Sci. U.S.A.* **104**, 14283 (2007).
8. M. N. Win, C. D. Smolke, *Science* **322**, 456 (2008).
9. Y. Wang, C.-G. Cheong, T. M. Tanaka Hall, Z. Wang, *Nat. Methods* **6**, 825 (2009).
10. M.-C. Gesnel, F. Del Gatto-Konczak, R. Breathnach, *J. Biomed. Biotechnol.* **2009**, 104853 (2009).
11. F. J. Isaacs *et al.*, *Nat. Biotechnol.* **24**, 545 (2006).
12. J. B. Lucks, L. Qi, W. R. Whitaker, A. P. Arkin, *Curr. Opin. Microbiol.* **11**, 567 (2008).
13. G. F. Joyce, *Angew. Chem. Int. Ed.* **46**, 6420 (2007).
14. C. A. Voigt, *Curr. Opin. Biotechnol.* **17**, 548 (2006).
15. A. S. Khalil, J. J. Collins, *Nat. Rev. Genet.* **11**, 367 (2010).

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EVOLUTION

Intermediate Steps

Damien P. Devos¹ and Emmanuel G. Reynaud²

Identifying the origin of the prokaryote-eukaryote transition has been a long-standing and contentious issue (1, 2) with numerous hypotheses. Over the last decade, “fusion hypotheses” have dominated the field (3). Many of these propose that the fusion of two prokaryotes accounts for the seemingly chimeric nature of the eukaryote gene set. Other theories propose that eukaryotes and archaea shared an ancestor that emerged from a bacterium (4). Thus, eukaryotes may be a mosaicism, combining features (including genes) inherited from a bacterial ancestor with those developed by an archaeal-eukaryotic ancestor before the split between eukaryotes and archaea. The hypotheses and debates about ancient cellular evolution continue as our knowledge of molecular and cellular mechanisms in all three domains of life expands (5). One intriguing curiosity is a “superphylum” of bacteria whose members display features of archaea and eukaryotes. What might these unusual organisms suggest in terms of evolution? It may be that their ancestor served as a “cauldron” for the evolution of eukaryotic and archaeal features, and that the superphylum is indicative of a path of intermediate steps between such an ancestor and an archaeal-eukaryotic ancestor (before the eventual split of the two domains).

The Planctomycetes, Verrucomicrobia, Chlamydiae (PVC) superphylum is an assemblage of Gram-negative bacterial phyla that is considered a monophyletic group in various phylogenetic trees derived from molecular sequence comparison (6). Some display genetic and cellular features that are unusual for bacteria but are characteristic of eukaryotes or archaea (see the figure). This mixture of phenotypes in current PVC members raises the hypothesis that they may be related to intermediate steps of eukaryotic and archaeal origin. Examination of this mixture of phenotypes can serve as a starting point for considering whether such features originated in a PVC-related ancestor from which the common eukaryotic and archaeal branch arose.

Two major steps in eukaryotic and archaeal evolution are loss of the peptidogly-

can-based cell wall of bacteria and replacement of the bacterial cytoskeletal protein FtsZ with eukaryotic tubulin. PVC members appear “intermediate” for both events. Some members synthesize peptidoglycan (Verrucomicrobia), whereas others do not (Planctomycetes and Chlamydiae) (7). Similarly, genomic information indicates that the last common ancestor of PVC bacteria possessed an FtsZ-based cell-division mechanism. However, Chlamydiae and Planctomycetes do not encode FtsZ in their genomes (7). The *Prostheco bacter* species (Verrucomicrobia phylum) have both FtsZ-like and tubulin-like genes (8, 9), which is unique among all bacterial phyla and has puzzled microbiologists.

Development of the endomembrane system that compartmentalizes the cytoplasm

Bacteria that also have features typical of eukaryotes and archaea may reflect a possible pathway in ancient cellular evolution.

has been crucial to eukaryogenesis (10, 11), but its origin is still mysterious. The functional and molecular similarities observed between the bacterial (Gram-negative) periplasm and eukaryotic endomembrane system (the space inside the organelles) underlies the hypothesis that the latter resulted from the internalization of the former by invagination of the bacterial inner membrane (10). Again, the membrane morphologies observed in PVC members appear intermediate. The PVC periplasm (or paryphoplasm) is usually larger with a more complex organization than the bacterial periplasm. Particularly, but not only, in the planctomycete *Gemmata obscuriglobus*, the inner membrane forms invaginations in the cytoplasm, sometimes in close contact with the DNA, in a way that appears to be dynamic and cell cycle dependent. The presence of ribosomes lining those invaginations is reminiscent of the eukaryotic rough endoplasmic reticulum, and vesicle-like structures are observed in *G. obscuriglobus*. This organization is sustained by proteins that may be related to those of the eukaryotic endomembrane system (11). PVC paryphoplasms may illustrate intermediate evolutionary attempts from the bacterial periplasm to the eukaryotic endomembrane system.

Periplasm internalization could provide a plausible scenario for the acquisition of mitochondrial precursors by eukaryotes (12). According to the endosymbiotic hypothesis, symbiosis and parasitism would allow progressive adaptation of both partners to each other. Some microbial symbionts or parasites reside in the host's periplasm. For example, *Bdellovibrio* bacteria are located in the periplasm of Gram-negative bacteria (13). The archaeon *Nanoarchaeum equitans* also localizes to the periplasm of the archaeon *Ignicoccus hospitalis* (14). Upon internalization of the periplasm, those guests would reside in the lumen of the internalized membranes.

Endocytosis development is linked to the development of the endomembrane system. Strikingly, the planctomycete *G. obscuriglobus* is the only bacterium in which a related process has been reported (15), again possibly reflecting an intermediate step between bacteria and eukaryotes. This proposal also offers a plausible intermediate for the development of the archaeal membrane that contains ether-linked lipids instead of the ester

PVC Superphylum

Features	Specific to	Found in
Compartmentalized cell plan (20)	Eu	Pl, Ve
DNA surrounded by membrane (21)	Eu	Pl
Condensed DNA (22)	Eu	Pl
Histone H1 (23)	Eu	Ch
Division by budding (24)***	Eu	Pl
Membrane coats (11)	Eu	Pl
Sterol (25)	Eu	Pl, Ch
Peptidoglycan loss (26)	Eu, Ar*	Pl, Ch
Proteic cell wall (27)	Eu	Pl
Ester and ether lipids (28)	Ar	Pl
FtsZ loss (7)	Eu, Ar**	Pl, Ch
Tubulin (8, 9)	Eu	Ve
C1 transfer (29, 30)	Ar	Pl
Endocytosis (15)	Eu	Pl

Superphylum features. The PVC superphylum of bacteria display features found in eukaryotes (Eu) and archaea (Ar). The particular feature is observed in at least one member of the indicated bacterial phylum. Methanobacteriales is an order of the Euryarchaeota that possess a pseudopeptidoglycan that differs from bacterial peptidoglycan (*). FtsZ is present in the main archaeal division of Euryarchaeota and absent from crenarchaeota (**). Budding also occurs in Proteobacteria (***). Planctomycetes (Pl), Verrucomicrobia (Ve), Chlamydiae (Ch).

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linkage found in bacterial and eukaryotic lipids (16, 17). The divergent planctomycete group, the Anammox bacteria, is so far, the only organism with membranes containing both ether- and ester-linked lipids.

The PVC bacteria are indeed a curiosity and their phylogenetic location in the Tree of Life is unclear. Although these features could be the result of lateral gene transfer events, convergence, or a complex universal ancestor, their intermediate nature can be considered a more parsimonious scenario. The possibility of a fusion scenario remains disputed (18). More evolutionary scenarios are likely to unfold—and be debated—as more bacteria and archaea are discovered and characterized. Although division into three domains of life remains the norm (19), the PVC superphy-

lum may reflect continuity between the three domains, blurring their distinction.

References

1. J. B. Dacks, W. F. Doolittle, *Cell* **107**, 419 (2001).
2. L. Aravind *et al.*, *Curr. Opin. Struct. Biol.* **16**, 409 (2006).
3. W. Martin, M. Müller, *Nature* **392**, 37 (1998).
4. C. de Duve, *Nat. Rev. Genet.* **8**, 395 (2007).
5. S. Gribaldo *et al.*, *Nat. Rev. Microbiol.* **8**, 743 (2010).
6. M. Wagner, M. Horn, *Curr. Opin. Biotechnol.* **17**, 241 (2006).
7. M. Pilhofer *et al.*, *J. Bacteriol.* **190**, 3192 (2008).
8. M. Pilhofer *et al.*, *Mol. Biol. Evol.* **24**, 1439 (2007).
9. B. Yee *et al.*, *BMC Evol. Biol.* **7**, 37 (2007).
10. C. De Duve, R. Wattiaux, *Annu. Rev. Physiol.* **28**, 435 (1966).
11. R. Santarella-Mellwig *et al.*, *PLoS Biol.* **8**, e1000281 (2010).
12. L. Sagan, *J. Theor. Biol.* **14**, 225 (1967).
13. A. Sánchez-Amat, F. Torrella, *Appl. Environ. Microbiol.* **56**, 2717 (1990).
14. H. Huber *et al.*, *Nature* **417**, 63 (2002).
15. T. G. A. Lonhienne *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **107**, 12883 (2010).
16. G. Wächtershäuser, *Mol. Microbiol.* **47**, 13 (2003).
17. J. Peretó *et al.*, *Trends Biochem. Sci.* **29**, 469 (2004).
18. P. Forterre, *Res. Microbiol.*, 10.1016/j.resmic.2010.10.005 (2010).
19. C. R. Woese, G. E. Fox, *Proc. Natl. Acad. Sci. U.S.A.* **74**, 5088 (1977).
20. K. Lee *et al.*, *BMC Microbiol.* **9**, 5 (2009).
21. J. A. F. erst, R. I. Webb, *Proc. Natl. Acad. Sci. U.S.A.* **88**, 8184 (1991).
22. J. A. Fuerst *et al.*, *FEMS Microbiol. Lett.* **166**, 29 (1998).
23. T. Hackstadt *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **88**, 3937 (1991).
24. K. C. Lee *et al.*, *BMC Cell Biol.* **10**, 4 (2009).
25. A. Pearson *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **100**, 15352 (2003).
26. E. König *et al.*, *Arch. Microbiol.* **138**, 200 (1984).
27. B. D. Kerger *et al.*, *Arch. Microbiol.* **149**, 255 (1988).
28. J. S. Sinninghe Damsté *et al.*, *Nature* **419**, 708 (2002).
29. L. Chistoserdova *et al.*, *Mol. Biol. Evol.* **21**, 1234 (2004).
30. M. Bauer *et al.*, *J. Mol. Evol.* **59**, 571 (2004).

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PHYSICS

Quantum Measurement and Control of Single Spins in Diamond

Gerard J. Milburn

One of the important advances in quantum physics during the past 25 years has been the development of an ability to make repeated measurements on a single quantum system. This capability began in ion-trapping experiments in the early 1980s (1) and was exploited in studies of the number of photons in a microwave cavity in the early 1990s (2). For quantum computing applications, solid-state implementations provide robust platforms, and phase measurements of single quantum bits in superconducting circuits have been made (3). Neumann *et al.* (4), in a recent issue,

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and Buckley *et al.* (5), on page 1212 of this issue, now report the repeated measurement of single spins in a particular type of defect in diamond—the nitrogen-vacancy (NV) color center—through changes in its fluorescence.

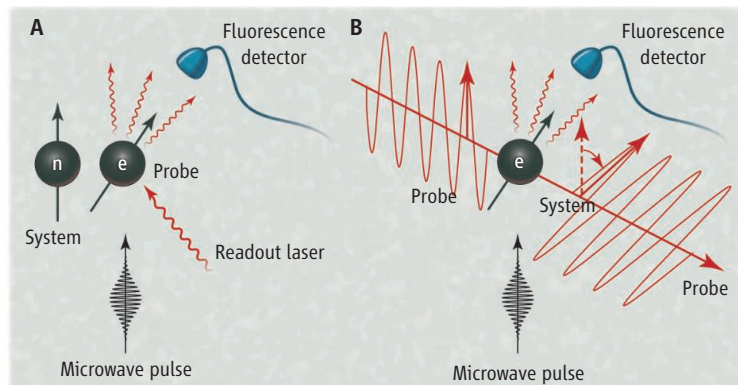
Quantum effects were discovered by making measurements on systems that are large ensembles of atomic systems where quantum and classical noise can be confused. For example, the explanation of the thermal spectrum of blackbody radiation necessarily combines Planck's hypothesis, restricting the energies available to an individual oscillator with classical thermodynamics in order to correctly describe measurements made on a macroscopic system. Even Schrödinger was confused on this point (6).

When repeated measurements are made

Changes in the state of a single spin at a defect in diamond, read out from optical measurements, could be used in quantum sensing and computing.

on one and the same quantum system, the need to correctly update the description of the quantum state of the measured system, conditional on the information obtained, becomes essential. In a long sequence of measurements, the state at the n th step can only be assigned if we know the initial state, the results of all preceding measurements, and the certainty (efficiency) of the measurement.

In a single spin- $\frac{1}{2}$ system prepared in an equal superposition of spin-up and spin-down, an efficient measurement allows the assignment of that state as spin-up. However, if the measurement has more uncertainty, the post-measurement state would need to contain some component of spin-down. The new assignment would need to reflect how good the measurement actually is.



Reading out the state of single spins. Two measurements of spin in a single nitrogen-vacancy (NV) diamond color center are depicted. (A) In the quantum nondemolition measurement (QND) scheme of Neumann *et al.*, the nuclear spin of a nitrogen atom is measured by first coupling it to an electron spin of the nearby NV center with an appropriate nuclear spin microwave pulse. The electron spin is then read out optically by means of electron spin-dependent fluorescence. The nuclear spin state is measured in a "single-shot" experiment with a probability of success greater than 90%. (B) In the scheme of Buckley *et al.*, the electron spin of the NV ground state is measured using the Faraday effect. The polarization of an optical probe field is rotated by an angle conditional on the electron spin. This is not a single-shot measurement but requires many samplings. A Hahn microwave pulse probes the decoherence of the electron spin caused by unwanted interactions of the electron spin with its environment.

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Repeated inefficient measurements on a single quantum system are easily described in the modern approach to quantum measurement that has its roots in the efforts to detect gravitational waves. In that setting, the idea of a quantum nondemolition measurement (QND) was developed. A sequence of QND measurements leads to a near-deterministic sequence of results. The noise added by a measurement does not dynamically feed back to the measured variable. Constants of the motion must be used as QND variables, and they need to remain constants of the motion when the measurement probe is coupled into the system. By contrast, in a non-QND experiment, any attempt to measure the position of a free particle very accurately would introduce a great deal of noise into its momentum. Changes in momentum couple back into the particle's position, so this noise contaminates future measurements of position.

The quantum control of single quantum systems requires three capabilities: state preparation, unitary control of the state, and quantum-limited measurement. In the case of NV diamond, the target quantum system is either a single electron or a nuclear spin. Usually, state preparation is achieved by passively cooling a system so that it relaxes to a ground state from which thermal excitation is improbable. Nuclear and electron spins in diamond can be polarized and remain so for sufficiently long times for these experiments, even at room temperature. Unitary control ensures that any operations on the system do not erase information about the quantum state.

A quantum-limited measurement is one that is efficient; that is, the signal-to-noise ratio is primarily determined by the quantum noise in the measured system itself and does not need many repeated trials to average out the noise added by the measurement apparatus—it yields a result in a “single shot.” Until now, however, there has been no way to make efficient quantum measurements on the spins in a single NV center. Both of the experiments of (4, 5) implement a QND measurement of a single spin—a nuclear spin in the case of Neumann *et al.*, and an electron spin in the case of Buckley *et al.*

In the Neumann *et al.* experiment, the measured system is a single nuclear spin of nitrogen at an NV center, while the first stage of the measurement probe is the associated electron spin (see the figure, panel A). When the NV center is excited by a microwave pulse, the nuclear spin can flip the associated NV electron spin, conditional on the nuclear spin being spin-down, while remaining unchanged itself. The electron spin is read out optically by means of spin-dependent flu-

orescence, very much like the earliest experiments on quantum jumps in ion traps. The Neumann *et al.* experiment approaches an efficient measurement.

In the Buckley *et al.* study, the measured system is the electron spin of the NV, and the probe is the polarization of a laser field (a Faraday effect; see the figure, panel B). This experiment measures the electron spin directly and also uses spin-dependent fluorescence. Their approach has the advantage of enabling very accurate characterization of the nature of the interaction between the spin and the light. However, unwanted interactions in the diamond crystal cause this spin not to be a strict QND variable, so their measurement is not efficient and causes unwanted decoherence. The Buckley *et al.* experiment required tens of thousands of repeated samplings, and the signature of unwanted decoherence is the decay of a coherent oscillation signal created by Hahn microwave pulses. In the case of the efficient measurement of Neumann *et al.*,

the signature of such unwanted interactions appear as “quantum jumps” in the observed fluorescence signal in a single trial.

These two experiments bode well for future quantum computing schemes that use NV diamond, but promise a more immediate spin-off for quantum sensing applications such as magnetometry. This capability could have an impact in biology and chemistry, especially if NV centers in diamond are used as nanocrystal probes (7). These experiments point to a future quantum sensing technology.

References

1. T. Sauter *et al.*, *Phys. Rev. Lett.* **57**, 1696 (1986).
2. M. Brune *et al.*, *Phys. Rev. Lett.* **65**, 976 (1990).
3. R. McDermott *et al.*, *Science* **307**, 1299 (2005).
4. P. Neumann *et al.*, *Science* **329**, 542 (2010) 10.1126/science.1189075.
5. B. B. Buckley *et al.*, *Science* **330**, 1212 (2010); 10.1126/science.1196436.
6. E. Schrödinger, *The Interpretation of Quantum Mechanics* (Ox Bow Press, Woodbridge, CT, 1995).
7. C. Bradac *et al.*, *Nat. Nanotechnol.* **5**, 345 (2010).

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NEUROSCIENCE

Lynx for Braking Plasticity

Michael J. Higley and Stephen M. Strittmatter

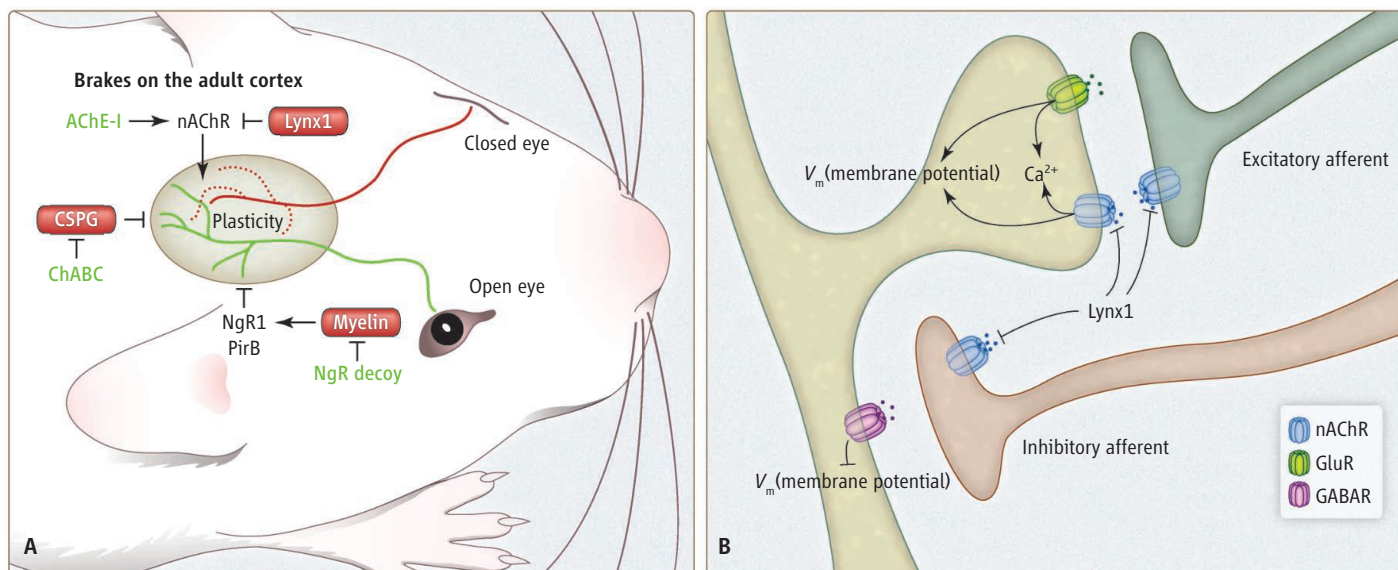
Knocking out the *Lynx1* gene restores plasticity in the visual cortex of adult mice.

The juvenile brain exhibits a high capacity for plasticity and repair that is severely restricted in adulthood. In young mammals, for instance, classic experiments have shown that closing one eye for several days (monocular deprivation) leads visual cortex neurons to shift their responses toward sensory inputs originating from the other, nondeprived eye (1). In adults, however, such plasticity in ocular dominance, while not eliminated, is strongly restricted. This knowledge gap has medical implications, because the restoration of juvenile plasticity in injured or dysfunctional adults has the potential to allow recovery of neurological performance. On page 1238 of this issue, Morishita *et al.* (2) identify one brake on visual cortex plasticity in adults: Lynx1, a protein that inhibits nicotinic acetylcholine receptors (nAChRs). By eliminating the gene that expresses Lynx1 in mice, the researchers were able to create adult animals that exhibited visual cortex plasticity similar to that

exhibited by juveniles.

Nicotinic receptors are present throughout the nervous system. They form “channels” that enable small ions to pass through neuronal membranes, and have “gates” that are opened by acetylcholine, a common neurotransmitter. Lynx1 is similar to snake venom proteins that inhibit nicotinic receptors, and deleting the *Lynx1* gene is known to increase cholinergic neurotransmission (the activity of acetylcholine) (3). Morishita *et al.* noted that, in juvenile mice, Lynx1 expression increases as the critical period for visual cortex plasticity closes. To better understand the role of Lynx1, they created knockout mice that lacked the *Lynx1* gene. Then, they used electrophysiological methods to measure the effect of monocular deprivation on neocortical ocular dominance (the eye preference of single cortical neurons) in both the knockout mice and wild-type mice that still expressed Lynx1. After 4 days of monocular deprivation, 60-day-old adult knockout mice exhibited plasticity that matched that of 30-day-old juvenile wild-type mice; in contrast, adult wild-type mice did not show such plasticity. Subsequent experiments with drugs that blocked nicotinic receptors produced results

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Easing the brakes on plasticity. (A) In juvenile wild-type mice, closing one eye for several days causes the loss of projections on the inactive pathway from the retina to the visual cortex (red lines for this polysynaptic pathway) and gains on the active pathway (green). In the adult, several pathways function as brakes on this plasticity in ocular dominance. Lynx1 inhibits nAChRs. Myelin inhibitors or CSPG-rich perineuronal nets also prevent rearrangement (11, 12). Specific

interventions can remove each of these brakes, including acetylcholinesterase inhibitors (AChE-I), NgR decoy protein, and chondroitinase (ChABC). (B) Nicotinic receptors are found on both pre- and postsynaptic elements of excitatory and inhibitory cortical neurons. Regulation of nAChRs by Lynx1 likely influences the balance of synaptic excitation and inhibition, and calcium influx through nAChRs may contribute to biochemical signaling pathways.

that were consistent with the hypothesis that Lynx1 acts by inhibiting nAChRs.

Previous studies have shown that blocking cholinergic signaling in the juvenile visual cortex during the critical period inhibits ocular dominance plasticity (4). In adults, enhanced cholinergic activity can promote activity-dependent plasticity in both auditory (5) and motor (6) regions of the brain. Morishita *et al.*, however, provide the first demonstration that acetylcholine signaling serves as a brake on adult visual cortex plasticity.

Other pathways have been implicated in preventing adult plasticity. Myelin proteins can act via the receptors NgR1 and/or PirB to inhibit the growth of neurites (7–9). Both NgR1 and PirB limit adult visual cortex plasticity to an extent similar to that shown for Lynx1 (10, 11). In addition, the perineuronal nets that surround inhibitory interneurons are rich in chondroitin sulfate proteoglycans (CSPGs). The development of these nets parallels both Lynx1 expression and the development of intracortical myelin sheaths, and CSPGs function as an additional brake on plasticity (12). Specifically, digesting CSPGs reestablishes ocular dominance plasticity in the adult brain (12). However, Lynx1 appears to be unique in its regulation of neurotransmission, in contrast to the anatomical role proposed for myelin and CSPGs.

Although inhibition of nicotinic signaling appears to be the primary mechanism by which Lynx1 regulates cortical plasticity, the specific cellular targets are not yet defined.

Nicotinic receptors are expressed on axonal terminals and postsynaptic membranes of both excitatory and inhibitory cells. Their activation likely alters the balance of synaptic excitation and inhibition, thereby changing the complex patterns of neuronal activity evoked by sensory inputs. Many nAChRs exhibit permeability to calcium ions, enabling them to contribute to calcium-dependent signaling pathways that may regulate synaptic plasticity. Lynx1 expression is also observed in subsets of inhibitory neurons (2), although it is not clear if this site is relevant for control of plasticity.

The mechanism of ocular dominance plasticity also is unclear. Given the persistent nature of cortical plasticity and the anatomical actions of NgR1, PirB, and CSPGs, Lynx1 and nAChR activation might modulate some aspect of intracerebral synaptic connectivity. Future studies will be required to elucidate whether this modulation involves changes in axonal branching, the formation or elimination of synaptic contacts, or simple changes in the efficacy of existing synapses. It also remains unclear whether nAChR function, myelin, and CSPGs act independently or cooperatively in influencing plasticity.

There is strong reason to believe that increasing adult brain plasticity can support neurologic recovery in a range of conditions (13). Morishita *et al.* examined how mice that experienced 2 weeks of juvenile monocular deprivation recovered from amblyopia (loss of visual acuity due to disuse). In adult mice

lacking Lynx1, simply reopening the closed eye caused electrophysiological signals to return to patterns indicating normal acuity. They obtained a similar degree of recovery from amblyopia in wild-type mice by administering an acetylcholinesterase inhibitor that increased acetylcholine transmission. Similar recovery through plasticity may underlie the beneficial effects of digesting CSPG or blocking NgR after spinal cord injury or stroke (14–16), and it will be of great interest to assess whether Lynx1 deletion or facilitation of nAChR activity enhances recovery from such neurological damage.

References and Notes

1. T. K. Hensch, *Annu. Rev. Neurosci.* **27**, 549 (2004).
2. H. Morishita *et al.*, *Science* **330**, 1238 (2010); 10.1126/science.1195320.
3. J. M. Miwa *et al.*, *Neuron* **23**, 105 (1999).
4. M. F. Bear, W. Singer, *Nature* **320**, 172 (1986).
5. R. C. Froemke *et al.*, *Nature* **450**, 425 (2007).
6. D. Ramanathan *et al.*, *J. Neurosci.* **29**, 5992 (2009).
7. J. K. Atwal *et al.*, *Science* **322**, 967 (2008).
8. A. E. Fournier *et al.*, *Nature* **409**, 341 (2001).
9. W. B. Cafferty, S. M. Strittmatter, *J. Neurosci.* **26**, 12242 (2006).
10. J. Syken *et al.*, *Science* **313**, 1795 (2006).
11. A. W. McGee *et al.*, *Science* **309**, 2222 (2005).
12. T. Pizzorusso *et al.*, *Science* **298**, 1248 (2002).
13. W. B. Cafferty *et al.*, *Trends Neurosci.* **31**, 215 (2008).
14. E. J. Bradbury *et al.*, *Nature* **416**, 636 (2002).
15. X. Wang *et al.*, *Ann. Neurol.* **60**, 540 (2006).
16. J. K. Lee, J. E. Kim, M. Sivula, S. M. Strittmatter, *J. Neurosci.* **24**, 6209 (2004).
17. S.M.S. is supported by grants from the NIH and the Falk Medical Research Trust. S.M.S. is a cofounder of Axerion Therapeutics, seeking to commercialize PrP- and NgR-based therapies.

10.1126/science.1198983

RETROSPECTIVE

Paul F. Barbara (1953–2010)

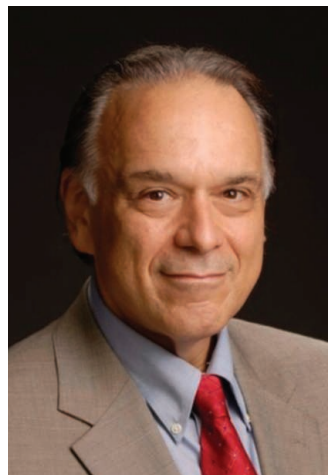
Peter J. Rossky¹ and Gilbert C. Walker²

Paul F. Barbara, 57, passed quietly on Sunday, 31 October 2010, from complications following cardiac arrest. He made deep contributions to understanding how molecular shapes and reactivities are induced by their environments. Paul's intense focus on understanding molecules at their most fundamental level had very early roots. After his election to the National Academy of Sciences, he was asked to comment on what brought him to science (1). He replied, "The first book I ever got was a book on molecules, and I think that was in fourth grade." He went on to talk about his early fascination with molecules: "I constantly imagined that they were there and that if you had an ideal microscope, you'd be able to see them and they'd be everywhere around you."

Around 1996, Barbara realized that early imagination and, in fact, embarked on studying individual polymer molecules, a focus that would dominate his scientific curiosity from then on. In groundbreaking work, his group developed the means to observe polymers on very small length scales and detect

These included proton and electron transfer reactions that are the essential steps in electrochemical processes and that make up the elementary chemical steps underlying the basic processes of photosynthesis as well as the metabolism of essentially all living species. Paul also developed a detailed description of solvated electron dynamics, a fundamental process associated with radiation chemistry. He and his co-workers achieved this through the development and application of novel ultrafast spectroscopies that could resolve different steps in these exceedingly fast processes.

Paul called himself an "instrument jock," and his mastery of state-of-the-art experimental tools was certainly an essential part of his impact. He was well known for casu-



He completed his Ph.D. at Brown University in 1978 and pursued postdoctoral studies at Bell Laboratories until 1980. He was a faculty member for 18 years at the University of Minnesota, where he was named 3M-Alumni Distinguished Professor of Chemistry. Moving in 1998 to Austin, he held the Richard J. V. Johnson–Welch Regents Chair in Chemistry at the University of Texas.

He received many awards and accolades throughout his career,

beginning with a Presidential Young Investigator Award in 1984. In 2009, he was awarded the E. Bright Wilson Award in Spectroscopy by the American Chemical Society, recognizing his innovative experimental probes of the dynamics of chemical processes using both ultrafast and single-molecule spectroscopies. In 2006, he was elected to the National Academy of Sciences. During his career, he published more than 200 influential and widely cited journal articles. He was also a mentor to more than 100 graduate students and postdoctoral research fellows; 34 are now professors at universities in the United States, Asia, and Europe, carrying forward his legacy in their own labs.

Paul's attitude toward science, while constantly focused on where careful measurements could resolve outstanding controversies, was playful and exuberant. When he had new data that inspired a new idea for how things worked, his excitement was childlike; infectious, it nucleated his colleagues to focus collectively on the grand problems that would change the way that people think. That excitement about new discoveries and the possibilities to upend the status quo with a new concept seemed to grow boundlessly over the many years that we knew and worked with Paul. He left us far too soon, and the scientific world will be too still without his presence.

Reference

1. K. Mossman, *Proc. Natl. Acad. Sci. U.S.A.* **104**, 17567 (2007).

When asked to comment on what brought him to science, Paul replied, "The first book I ever got was a book on molecules, and I think that was in fourth grade."

the weak light needed to probe their molecular arrangements. They could pick out individual structural arrangements and see how these affected the observed molecular behavior in complex environments. They studied not only synthetic polymers, such as the candidates for components in plastic solar cells, but also biopolymers. Stimulated by his collaborator, biological chemist Karin Musier-Forsyth, his group performed elegant studies on the sequence of molecular events occurring during reverse transcription in HIV.

Before studying single molecules, Paul had already made deep and lasting contributions to the understanding of condensed phase molecular dynamics in complex chemical contexts that are ubiquitous in both solution chemistry and biological chemistry.

ally disassembling finicky instruments in front of their horrified users whose careers depended on their instruments. They would then stare in disbelief as he casually put the apparatus together again in a new way that worked much better. But the vital feature that made Paul Barbara a unique force in everything he did was that Paul was a dynamo. A dynamo not only of energy, and there was ceaseless energy, but also of ideas. Ideas for experiments—and for theories—as well as for conceptual molecular interpretations of physical behavior that were never confined by conventional wisdom. He would call the lab from home into the wee hours, night after night, eager to discuss the newest data coming from those finicky instruments, encouraging his students, while reminding them of the control experiments that would make the labors worthwhile.

Paul grew up in New York City and received his B.S. in chemistry at Hofstra University in Long Island, New York, in 1974.

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SPORE* SERIES WINNER

Got a Question? "Ask A Biologist"

Charles Kazilek

Adjusting her headphones, fifth-grader Itzany Mendez carefully reads through her handwritten list to select her next interview question. She fixes Rebecca Clark, a doctoral student in the School of Life Sciences at Arizona State University (ASU), with a curious look (see the first figure). Mendez's question reflects the perspective and natural observation skills found in a young scientist: "Why do ants huddle up like penguins?" she asks. "That's a great question," Clark says, as she launches into a short discussion of animals and social groups.

Mendez is one of three fifth-graders gathered in the bright blue- and green-painted Grass Roots Studio, the recording site for the Ask A Biologist podcast. These students are winners of the Ask A Biologist podcast cohost contest, whose high point is this moment, where they cohost a podcast of their own and interview an ASU scientist. Along with their parents and teachers, they have also had tours of laboratories, constructed ant farms from recycled music CD cases, peered inside leaf cutter colonies to see carefully tended fungal gardens, discovered wicked plants, and observed how birds use their feathers. Most importantly, the students have discovered what scientists do on a daily basis, and how they can fill those same shoes.

Mendez's time with Clark opened the world of science in a most personal way. Most online science education portals cannot achieve this one-on-one exchange and also scale up to reach millions. However, the Ask A Biologist Web site (askabiologist.asu.edu) which hosts the podcast and the podcast contest, has been on this path in multiple forms since 1997.

Ask A Biologist was originally created as a vehicle to host an online question-and-answer (Q&A) feature for kids. Since its inception, the Web site has answered more than 25,000 queries from students, teachers, and lifelong learners. Users come from around the globe to ask some of biology's most perplexing questions. Why do some plant leaves turn red or yellow in the winter? Why are fingers wrinkled after a long bath?

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*SPORE, Science Prize for Online Resources in Education, www.sciencemag.org/special/spore/.



I. Mendez learns some ant facts from R. Clark (top) and asks questions during a podcast recording session (bottom).

Teachers, both formal and informal, are also a key portion of the audience. Instructors of K–12 students can find confirmation or advice about a range of topics in biology. In some cases, it is unexpected results from an experiment that send a teacher to Ask A Biologist for clarification or advice. Sometimes it's a complicated topic, such as whether genetically modified food crops affect migrating butterflies, that leads them to the Web site. The addition of the Ask A Biologist podcast in 2006 gives teachers the ability to bring scientists' voices into the classroom to mesh with the day's lessons. Parents and grandparents can use the Web site and podcast program to help teach their young charges or to satisfy their own curiosity.

As Ask A Biologist has grown, its content has also adapted to visitors' needs. Although search engine tools have improved and made it easier to find information, search engine results often return multiple answers to a question, sometimes with conflicting, incorrect, or out-of-date information. Resolving these conflicts requires more than a search engine; it takes the human touch. This is the power of putting the working scientist in touch with children and the public. The ASU School of Life Sciences houses more

An educational Web site adds new twists to learning by promoting direct conversations between scientists and the public.

than 100 faculty and 240 graduate students. This pool of expert volunteers—with their range of experiences, challenges, and scientific insights—is an immense resource. Their diverse backgrounds often provide the perspective needed to resolve contradictions and inadequate answers that pop up in Web searches.

The Q&A process work begins with a simple e-mail form. Through an established work flow, Ask A Biologist receives and distributes questions to volunteer scientists in the School of Life Sciences and other institutions. This ensures that questions are directed toward the best possible experts. It also allows the opportunity for review of the answer to make sure it is grade appropriate. The form uses a generic e-mail address that protects volunteers from being overwhelmed by questions. An off-line database of past questions and answers can be used for repeat questions. However, off-line materials usually require modifications, to address the grade level of the person submitting the question, and revisions to include the most current scientific findings. Maintaining the database off-line also keeps outdated information off the Web, eliminating the possibility that stu-



Zoom Gallery.

PHOTO CREDITS: PHOTOGRAPHS AND ILLUSTRATIONS/SCHOOL OF LIFE SCIENCES VISUALIZATION LABORATORY

Downloaded from www.sciencemag.org on November 25, 2010

dents or teachers will confound current information with out-of-date replies.

The Q&A feature has also served the secondary role of advancing the Web site's development. Directly linked with users, this tool has guided the creation of content. Once a single page, the Ask A Biologist Web site now has more than 2500 pages of materials. Many of the pages were "asked" for as a direct result of questions sent via the Web site form. For example, the coloring page section was developed in response to parents and grandparents searching for activities for young students who are at pre- or early reading levels. The Quizzes and the Mystery Image gallery came about in response to student requests for ways to test their comprehension of articles and a desire to explore the microscopic world.

Of the 25,000 questions posed to Ask A Biologist, roughly 25% are career related, resulting in the development of our "Meet Our Biologists" profiles. Students ask questions such as "What is it like to put on a lab coat and work in a laboratory?" and "What is it like to travel to Africa or the Arctic to study plants and animals?" Other times, children are looking for academic guidance and ask, "What are the best classes to take in high school if you want to be wildlife biologist?" and "Do I need to take math in school if I want to be a biologist?" A glance through the Ask A Biologist podcast collection tells the tale clearly: There is no one way to become a scientist and no single type of person who one has to be in order to be passionate about science.

Having a direct link with the public has been invaluable in the development of Ask A Biologist materials. One key lesson learned through interaction with teachers is that even though the Web is a powerful tool, content that can only be used with a computer places limits on its use. For this reason, the Web site includes materials that can be used on- and off-line, or a combination of both. Teachers are also looking for learning tools that include data sets. With these two priorities in mind, Ask A Biologist has invested in development of materials that are flexible in how and where they can be used.

One such activity is the Virtual Pocket Seed Experiment. This activity provides options for exploring seed germination, energy, gravitropism, and the scientific method both online and as a hands-on experiment. Online there is a pictorial data set involving various seed germination treatments. To help engage students in the activity, time-lapse animations of each seed treatment are included. The companion



Mysterious World of Dr. Biology.

ion Pocket Seed Packet can be downloaded and used to run the experiment as a hands-on activity in the classroom or at home. This places experimental control in the hands of students. The flexibility of the activity allows teachers to select which options best fit their students' needs as well as their access to technology. The popularity of this activity is reflected in the Web site analytics and usage requests submitted through the online permission form. It is downloaded and used by hundreds of thousands of students and teachers yearly.

Ongoing dialogue with the public has also led to the development of other media, including coloring pages, worksheets, word puzzles, and quizzes. All of these are tied to online stories and activities. Most recently, Ask A Biologist has added companion video channels on Vimeo, YouTube, and TeacherTube. The Image Gallery has added the biology-themed computer wallpaper and the popular Zoom Galleries, which offer the opportunity to explore the macro and microscopic world (see the second figure). Using a simple microscope-like interface, visitors can explore the beauty and structure of feathers or take an ultra-close view of pollen grains.

In addition to focusing on science education, Ask A Biologist recognizes the need for expanding student language skills to incorporate new ideas and concepts. Capitalizing on the popularity of comic book-based content, the Mysterious World of Dr. Biology was developed (see the third figure).

This activity offers a collection of biology-themed clip-art. Students fill in blank text bubbles and chose panels to create their own stories. The comic-book theme allows children to partner creative thinking with written language skills development around a biology-based theme (1).

What is next for Ask A Biologist? As the Internet becomes increasingly accessible on a global level, it is important to provide content in languages other than English. Using its established volunteer model, the program is adding French and Spanish translations of its content to the Web site. New partnerships are also being formed to expand content-rich sections within the Web site. For example, Ask A Biologist is working with the Arizona Science Center to build and host a Web version of their Body Depot gallery program (2). The project introduces students, ages 8 to 14 years, and their teachers to three biomedical research areas inspired by National Institutes of Health's Roadmap (3) for Medical Research: Biological Pathways, Bioinformatics, and Nanomedicine, by using the metaphor of a hardware store to explain how the body maintains and repairs itself.

As access to the Internet continues to grow and educators and content developers refine online tools, the future of science education seems bright. No other tool has been as powerful as the Internet for putting science education directly in users' hands, in multiple formats that can excite discussion and critical thinking. Ask A Biologist plans to continue offering students, teachers, parents, and life-long learners access to the latest in scientific trends and technology while maintaining the link between the public and working scientists. This social component of Ask A Biologist has been a key to its past success and will remain a vital part of future development.

References and Notes

1. Teacher Information, Ask A Biologist; <http://askabiologist.asu.edu/teacher-information>.
2. Body Depot is part of the NIH-funded program, Framing New Pathways to Medical Discovery for Families, Students, and Teachers.
3. New Pathways to Discovery, The NIH Common Fund, <http://nihroadmap.nih.gov/newpathways/>.
4. Ask A Biologist is funded in part by a grant from the National Science Foundation and the National STEM Distributed Learning program (award 0840788). I thank J. Sahertian, S. Deviche, J. Mayfield, and J. Baxter for photographs and illustrations. Grass Roots Studio photo by Actual Films.
5. Charles Kazilek is the director of Technology Integration and Outreach for the ASU School of Life Sciences, a division of the College of Liberal Arts and Sciences. He is also the creator and caretaker of the Ask A Biologist program and Web site. Mr. Kazilek would like to thank Margaret Coulombe for her invaluable help in reviewing and editing this essay.

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SCIENCE POLICY

Aided by Early Career Scientists, U.S. Plans for Climate Adaptation

In the summer of 2009, Ilya Fischhoff was in Kenya, studying zebras and their interactions with the predators, livestock, and people who share an environment that was, at the time, parched by drought. Within weeks after his return to the United States, Fischhoff was looking at adaptation from a new angle, contributing to a report on how the U.S. government can help the nation and other countries prepare for climate change.

The report, requested by President Barack Obama, for the first time offers recommendations to guide the federal government's climate change adaptation efforts. Fischhoff and five other AAAS Science & Technology Policy Fellows joined over 300 federal administrators and staff in the multi-agency effort, with much of their work focused on ensuring that scientific information is readily available to U.S., state, and local policy-makers and the public.

"People can make good decisions if they have scientific information that they trust and that is relevant," he said. "For that trust and relevance to be there, I think it is important for citizens and scientists to be partners. That way, we create knowledge that is useful and empowering to everyone."

The "Progress Report of the Interagency Climate Change Adaptation Task Force" was released 14 October, a year after the White House launched the effort. Based on the work of more than 20 federal agencies, and informed by 35 listening sessions and outreach events, the report reflects the growing awareness that while reducing greenhouse gases remains a priority, the nation must be ready as changes already evident in the climate increase and intensify (see the report at <http://go.usa.gov/CIP>).

As atmospheric concentrations of greenhouse gases have risen, the year-round average U.S. temperature has risen 2°F in the past 50 years. Wet areas of the country are expected to get wetter, and dry areas drier. "Climate change affects human health, water and energy supplies, food production, coastal



Planning for change. Lake Mead's "ring" reflects persistent drought in parts of the American West. A new federal report, with input from AAAS S&T Policy Fellows, says U.S. agencies should help policy-makers at every level adapt to long-term challenges that may be linked to climate change.

communities, [and] ecosystems," the report says. While future changes are difficult to predict, it adds, research indicates they "will be significant."

The task force is cochaired by Nancy Sutley, chair of the White House Council on Environmental Quality; Jane Lubchenco, administrator of the National Oceanic and Atmospheric Administration (NOAA); and Shere Abbott, associate director for environment in the White House Office of Science and Technology Policy (OSTP).

The AAAS Science & Technology Policy Fellowships, founded in 1973, offer early-through senior-career scientists and engineers positions in Congress or federal agencies. Fischhoff, for example, was assigned for 1 year to the U.S. Agency for International Development in the Executive Branch, and this fall began a congressional assignment.

Other Fellows who worked with the Task Force included: marine biologist Laura Petes and biologist Christine Jessup, both assigned to NOAA; sociologist Sabrina McCormick and ecologist Jdsen Bruzgul at the Environmental Protection Agency; and neuroscientist Sarah Carter at OSTP. (After completing their Fellowships, Jessup is now at the National Institutes of Health, and Carter remains at OSTP.)

The Fellows contributed to various task force work groups, but all of them participated on the science work group, which focused on improving the integration of science into adaptation policy at every level. The science work group's recommendations were based in part on six listening sessions, some by conference call, during which state and local officials, transportation managers, and others located from Alaska to the Gulf Coast described their adaptation planning needs.

The group found that while adaptation planning has already begun in many locations across the United States, barriers still exist, Petes explained. Local and state leaders are looking to the federal government for technical support and guidance—and the flexibility to shape their own plans. "We need to improve access to science in order to help individuals and institutions make informed adaptation decisions," she said. "Given that most adaptation actions are local, preparing the nation for the impacts of climate change will require integrated approaches and partnerships."

Abbott, formerly the AAAS chief international officer, said a key task force recommendation is to "use the science we have today more effectively, even as we work to fill knowledge gaps." She praised the Fellows for bringing "energy, focus, and scientific rigor to the process."

"Planning for a changing climate requires access to scientific information that people can understand and use," said Lubchenco, who served as AAAS president in 1996. She thanked the Fellows for their contribution, adding: "We can expect to see these young people at the forefront of the ongoing effort to communicate best-available science to those who need it the most."

The task force will deliver another report to Obama in October 2011 to detail progress in implementing the recommendations.

Middle East Scientists, AAAS Work for Greater Regional Collaboration

Science research and education thrive in innovative programs throughout the Middle East and North Africa, but these efforts are too often isolated and could benefit from stronger regional partnerships, experts said at two recent meetings held in Amman, Jordan, and organized by AAAS.

One conference discussed ways to identify and nurture math and science talent among pre-college students in the region, while the second conference promoted international collaboration in the biosciences. Despite their different subjects, the meetings shared a common theme: Researchers are eager to develop regional networks that will allow them to collaborate and compete globally.

The meetings fostered new working relationships between scientists, educators, and research institution administrators from different countries, a goal that has been hampered by a lack of funding and a sparse history of collaboration, participants said.

It was “immediately apparent how much open dialogue between regional and international scientists was effective in establishing new networks for scientists in areas of training and research,” said Saied Jaradat, director of the H.R.H. Princess Haya Biotechnology Center at the Jordan University of Science and Technology.

“We thought of joint research projects, site visit exchanges, sharing some data, and working together as a team,” said Abdullah Aljughaiman, director of Saudi Arabia’s

National Research Center for Giftedness and Creativity, who attended the talent conference. “These actions would help all of us to better achieve our goals and better use our resources.”

The meetings represent the most recent efforts by AAAS in its engagement in the Middle East and Northern Africa region. In early 2007, AAAS helped organize a landmark meeting in Kuwait that brought 200 women scientists

and engineers from the Arab world together with U.S. women who were leaders in science, business, education, and government. Norman P. Neureiter, while director of the AAAS Center for Science, Technology, and Security Policy, was part of a high-level delegation of scientists and engineers that visited Iran later that year. And in 2009, top AAAS officials joined a small, high-level delegation that visited Syria and met with President Bashar al-Assad and others for discussions focused on science, education, and development.

Neureiter, now a senior adviser at the center, spoke at the 27 to 29 September talent meeting in Amman with conference director Florence Fasanelli, associate program director in Education and Human Resources at AAAS. “The spontaneous interaction among people who work with gifted young people in the region, most of whom had not known each other before, was really inspiring,” he said.

The talent conference, co-organized by AAAS and Jordan’s Royal Scientific Society with support from the John Templeton Foundation, included more than 90 scientists and policy-makers from 17 countries. They discussed math and science enrichment programs, entry into the International Mathematics Olympiad, and barriers to identifying gifted youth in the region.

“Our goal must be to captivate talent and to nurture entrepreneurship, inquiry and skill,” said Her Royal Highness Princess Sumaya bint El Hassan of Jordan, in her



Commitment to excellence. Her Royal Highness Princess Sumaya bint El Hassan of Jordan said the region must do more to prepare its young scientists for the global knowledge economy.

opening speech at the conference. “We must offer our young scientists nothing less than the complete nurturing environment that they deserve.”

The 2 to 6 October biosciences meeting was cohosted by Jaradat and Gerald Epstein, director of the AAAS Center for Science, Technology and Security Policy. It drew more than 60 researchers, university administrators, and laboratory heads from 15 countries. The conference, sponsored by the U.S. Department of State, was organized in part by Kavita Berger, the center’s associate program director; and Gwenaële Coat, a senior program associate. Joanne Carney, director of the AAAS Center for Science, Technology and Congress, spoke in a session on communicating with policy-makers.

In far-ranging talks about research standards, private-public research partnerships, and infectious disease projects, the participants identified a number of barriers to international and regional collaboration. Among them: disparate government investment, bureaucratic obstacles, and different regulatory environments. AAAS will hold three additional meetings in the region to expand on the themes emerging from the conference, Epstein said.

“Even within institutions in the region, there isn’t much history of collaboration,” Epstein noted. “But if internal and regional partnerships can be created, these institutions would be better positioned to collaborate internationally.”

—Becky Ham

2010 ELECTION

A Call for Nominations

AAAS members may suggest nominees (including themselves) for president-elect and the Board of Directors for election in the fall of 2011. For a list of this year’s candidates, see AAAS News & Notes in the 29 October 2010 issue of *Science*; for a list of current Board members, go to www.aaas.org/aboutaaas/organization/board.shtml. Please send the suggested nominee’s curriculum vitae no later than 15 May 2011 to Gretchen Seiler, AAAS Executive Office, 1200 New York Ave., N.W., Washington, D.C., 20005. Suggested nominees will be considered by the AAAS Committee on Nominations at its winter meeting.

SUSTAINABLE DEVELOPMENT

Romain Murenzi to Head Sustainability Center

Romain Murenzi, a Rwandan physicist who helped shape his nation's acclaimed science-for-development strategy, has joined the AAAS International Office as director of the Center for Science, Technology, and Sustainable Development.

The center should be a hub where developing nations, scientists, and development agencies and banks come together, Murenzi said in an interview. That could make it a catalyst that helps marshal science, engineering, and technology to address challenges ranging from hunger and a lack of drinking water to science education and environmental threats.

"Science cooperation with the developing world is a central element of AAAS's mission, and in Romain Murenzi, we have an accomplished leader," said Alan I. Leshner, chief executive officer of AAAS and executive publisher of *Science*. "He has a vision for how science and technology can help build even the poorest nations—a vision informed by his experience as a scientist, teacher, and policy-maker. We believe he will have a tremendous impact."



Romain Murenzi

Murenzi traveled a remarkable route to AAAS. He was born in Rwanda and taught math in the neighboring nation of Burundi. After receiving his Ph.D. in physics at the Catholic University of Louvain in Belgium, he joined the faculty at Clark Atlanta University in Georgia. In 1999, he became chair of the Physics Department.

In 2001, he returned to Rwanda to join President Paul Kagame's efforts to help the impoverished nation recover from a 1994 genocide that left some 800,000 people dead, mainly ethnic Tutsis, as well as politically moderate Hutus.

Kagame saw science and technology as drivers of human and economic development. With Murenzi in ministerial posts overseeing education, scientific research, and communication technology, Rwanda made dramatic strides in school enrollment, health care, food production, and wireless and fiberoptic networks. Today, it is a key center in Africa's increasingly vibrant economy.

Find a podcast with Romain Murenzi at www.aaas.org/go/murenzi.

COMMUNICATION

Winners Named in AAAS Kavli Science Journalism Awards

Probing environmental reports and intriguing memory studies were among the 2010 winners of the prestigious AAAS Kavli Science Journalism Awards.

Large Newspaper—(Circulation $\geq 100,000$): Charles Duhigg, *The New York Times*, for "Toxic Waters," 17 December 2009, 13 September 2009, and 23 August 2009.

Small Newspaper—(Circulation $< 100,000$): Hillary Rosner, *High Country News*, for "One Tough Sucker," 7 June 2010.

Magazine: Steve Silberman, *Wired*, for "The Placebo Problem," September 2009.

Television—(Spot News/Feature Reporting, ≤ 20 minutes): Sarah Holt, NOVA scienceNOW, for "How Memory Works," 25 August 2009.

Spot News Certificate of Merit: Vince Patton, Nick Fisher, Michael Bendixen and Todd Sonflieth, Oregon Public Broadcasting, for "Murre and Eagles" and "Pygmy Owls," 4 February 2010 and 18 February 2010.

Television—(In-Depth Reporting, > 20 minutes): Alan Alda, Graham Chedd, Larry Engel, and Jared Lipworth, THIRTEEN, in association with WNET.ORG, for "The Human Spark," 6, 13, and 20 January 2010.

Radio: Richard Harris and Alison Richards, NPR, for "Follow the Science: Calculating the Amount of Oil and Gas in the Gulf Oil Spill," 14, 20, and 28 May 2010.

Radio Certificate of Merit: Gabriel Spitzer, WBEZ, for "Researchers Probe How Music Rewires the Brain," 10 September 2009.

Online: William Saletan, Slate, for "The Memory Doctor," 4 June 2010.

Children's science news: Cody Crane, *Science World* (Scholastic), for "Learning from Bears," "Real-Life Bloodsuckers," and "Saving the Ozone Layer," 1 February 2010; 26 October 2009; and 7 September 2009.

The awards, administered by AAAS since their inception in 1945, go to professional journalists for distinguished reporting for a general audience. The Kavli Foundation, based in Oxnard, California, provided a generous endowment for the program in 2009.

Independent panels of science journalists pick the winners, who will receive \$3000 and a plaque at the 2011 AAAS Annual Meeting in Washington, D.C.

—Earl Lane

SCIENCE & DIPLOMACY

Neureiter Awarded Japan's Order of the Rising Sun

Norman P. Neureiter, the first director of the AAAS Center for Science, Technology and Security Policy, has received one of the highest honors awarded by the Japanese government, for his career contributions to science and technology cooperation between Japan and the United States.

Neureiter, now a senior adviser to the AAAS Center, accepted the Order of the Rising Sun, Gold and Silver Star decoration in a ceremony at the Imperial Palace in Tokyo on 5 November 2010. The investiture ceremony was followed by a reception with Emperor Akihito.

"We are delighted that Japan has seen fit to honor Dr. Neureiter's global science leadership in this very prestigious way," said Alan I. Leshner, chief executive officer of AAAS and executive publisher of *Science*. "He is a fine colleague who leads both intellectually and through his own extensive international activities."

Neureiter started working with Japan in 1963, when he became the first permanent U.S. program director for the U.S.–Japan



Norman Neureiter

Cooperative Science Program that was initiated under President John F. Kennedy. From 1989 to 1994, he lived in Tokyo and served as vice president of Texas Instruments Asia.

In 1994, Neureiter was asked by the White House Office of Science and Technology Policy to chair the U.S. side of an advisory committee established under the U.S.–Japan Science Cooperation Agreement. He served as U.S. cochair of that committee, the Joint High-Level Advisory Panel, until 2000.

Neureiter said the award ceremony and reception gave him and his wife, Georgine, "a poignant sense of the rich historical traditions of Japan, while recognizing at the same time Japan's own unique contributions to cutting-edge science and technology."

Neureiter, who speaks Japanese, visits Japan at least once a year for professional activities. In October, he spoke about science diplomacy at the 50th anniversary celebration of the U.S. National Science Foundation's Tokyo office.

—Earl Lane

From the Connectome to the Synaptome: An Epic Love Story

Javier DeFelipe

A major challenge in neuroscience is to decipher the structural layout of the brain. The term “connectome” has recently been proposed to refer to the highly organized connection matrix of the human brain. However, defining how information flows through such a complex system represents so difficult a task that it seems unlikely it could be achieved in the near future or, for the most pessimistic, perhaps ever. Circuit diagrams of the nervous system can be considered at different levels, although they are surely impossible to complete at the synaptic level. Nevertheless, advances in our capacity to marry macro- and microscopic data may help establish a realistic statistical model that could describe connectivity at the ultrastructural level, the “synaptome,” giving us cause for optimism.

What mysterious forces precede the appearance of the processes [dendrites and axon], promoting their growth and ramification, provoking the coherent migration of the cells and fibres in predetermined directions, as if obeying a wise architectonic plan, and finally establishing those protoplasmic kisses, the intercellular articulations [synapses] that appear to constitute the final ecstasy of an epic love story? —Santiago Ramón y Cajal (*Recuerdos de mi Vida*, Moya, Madrid, 1917)

Early Circuit Diagrams: The Neuron Doctrine and the Dynamic Polarization of Nerve Cells

From the outset of Cajal's studies in 1888 with the revolutionary method of Golgi, he provided strong support for his belief that dendrites and axons end freely in the nervous system and that they communicate by contact (1). This hypothesis contrasted with the most prevalent idea at the time, developed by Gerlach, that the elements of the nervous system formed a continuum (Fig. 1).

The existence of a continuous network would more readily explain the flow of currents, but how could this be possible through an infinitely interrupted and fragmented nervous system (Fig. 2)? Cajal proposed in 1891 that neurons showed a morphological and functional polarization in such a way that neurons could be divided in general into three functionally distinct regions: a receptor apparatus (formed by the

dendrites and soma), the emission apparatus (the axon), and the distribution apparatus (terminal axonal arborization). This idea was based on the direction followed by impulses in cer-

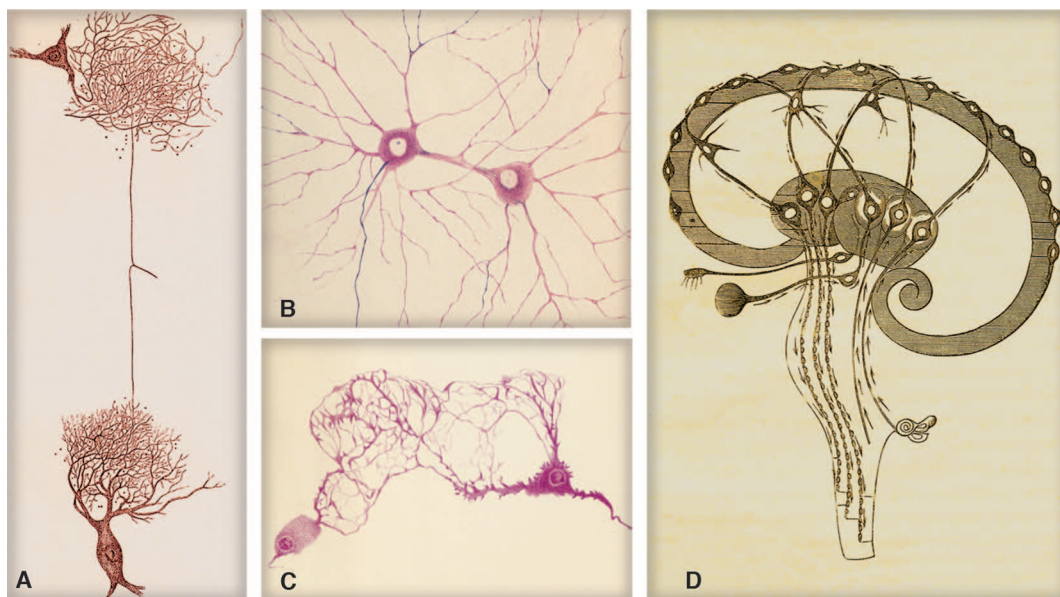


Fig. 1. In the reticular theory, the brain was thought to be made up of a mesh of nerve cell processes. (A) A drawing from Gerlach from 1872 showing two nerve cells from the spinal cord of the ox. According to Gerlach, nervous activity was driven through a network of neural elements formed by axons (axonal network) and dendrites (dendritic network). (B and C) Drawings of ganglion cells from the human retina and of cells from the dog's gall-bladder ganglia produced by Dogiel in 1893 and 1899, respectively. (D) Luys' schematic illustration from 1878 showing the processing of sensory-motor cerebral activity. [From (26)]

tain regions of the nervous system in which their activity clearly reflected the anatomical routes the impulse followed, such as in the visual and olfactory systems (Fig. 3, left). Cajal's new ideas about the connections between neurons led to novel theories on the relationship between neuronal circuits and brain function. Indeed, this hypothesis gave rise to a new era in neuroscience and to the tracing of the first point-to-point connectivity maps of the nervous system (Fig. 3, right).

Visualization of the Synaptic Cleft: The Discovery of the Physical Separation Between Neuronal Processes

In the 1930s, it had been shown histologically that the terminal axonal bouton or synaptic specialization was separated by a “membranous synaptic barrier” (2, 3). The large number of studies demonstrating this phenomenon resolved this issue for the vast majority of scientists; therefore, they turned their interest to other aspects of synaptic structure. At the areas of contact between the axon terminal and the soma or dendrite, only one membrane was visible (the synaptic membrane), presumably because the membrane of the pre- and postsynaptic elements were so close together that only a single membrane could be distinguished. Thanks to the introduction of transmission electron microscopy (TEM) in the 1950s, along with the development of methods to prepare nervous tissue for ultrastructural analysis, the nature of synapses was examined, confirming a critical aspect of the neuron theory: The pre-

synaptic and the postsynaptic elements in both invertebrate and vertebrate nervous tissues are physically separated by a space of approximately 10 to 20 nm, the synaptic cleft (4, 5). This essentially confirmed the observations and theories of Cajal.

Exceptions that Challenge the Neuron Doctrine and the Law of Dynamic Polarization

The appearance of new techniques to study the nervous system at the anatomical, physiological,

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and molecular levels has helped to identify many exceptions that challenge the neuron doctrine and the law of dynamic polarization. For example, axons have been found to form synapses with other axons (axo-axonal synapses), and presynaptic elements are not necessarily axonal but may come from dendrites or the soma. Furthermore, neurons are not only connected by chemical synapses but may also be coupled electrically through gap junctions (6). Furthermore, the transmitter released at synaptic or nonsynaptic sites may diffuse and act on other synaptic contacts or on extrasynaptic receptors (7). Finally, it has been proposed that glial cells are involved in information processing through their bidirectional signaling with neurons (8). These different features introduce further complexity into the system, making it even more difficult to establish the correct flow of information and wiring of neural circuits. Nevertheless, chemical axodendritic synapses

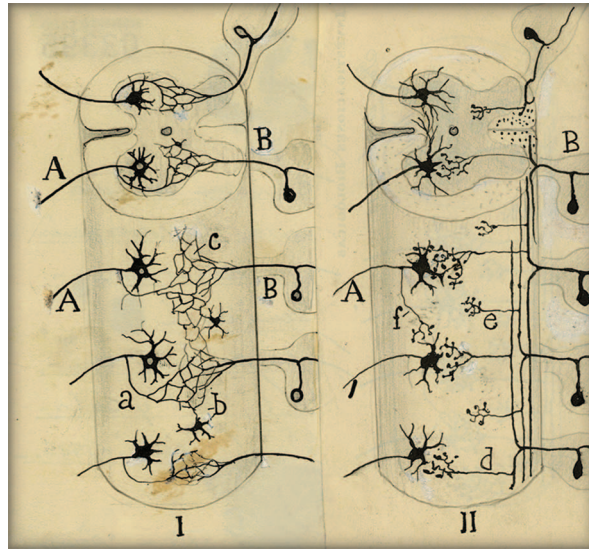


Fig. 2. Cajal's drawing from 1917 to explain the differences between the neuron and the reticular theories (27). The figure legend states: "Scheme to compare Golgi's concept regarding the sensory-motor connections in the spinal cord (I) with the results of my own studies (II). A, anterior roots; B, posterior roots; a, collateral of a motor root; b, cells with a short axon that, according to Golgi, would intervene in the formation of the network; c, diffuse interstitial network; d, our long collaterals in contact with the motor cells; e, short collaterals; f, collateral of a motor root." [Copyright Herederos de Santiago Ramón y Cajal]

are by far the most common type of synapse (followed by axosomatic synapses), at least in mammals. Other types of synapses are not found in all regions of the nervous system, and when they are present, they are usually only established between specific types of neurons. Therefore, the idea of the nerve cell as an independent cellular unit, with an overall polarization to mediate its input-output functions, continues to be one of the foundations on which our concept of nervous activity is based (9).

Bridging the Gap Between the Connectome and the Synaptome

The term connectome was introduced to define the connection matrix in the human brain in analogy to the human genome (10), although this term was rapidly adopted to describe maps of neural circuits in general (11, 12). However, it is often forgotten that the connectivity of the nervous system can be analyzed at three quite distinct levels: (i) macroscopically, focusing on major tract connectivity, for example, by examining images of the whole brain (or of large brain regions), which can even be performed *in vivo* by magnetic resonance imaging (MRI) or other techniques; (ii) at an intermediate resolution, as can be achieved by light microscopy, which in addition allows putative synaptic contacts to be mapped; and (iii) at the ultrastructural level, which can only

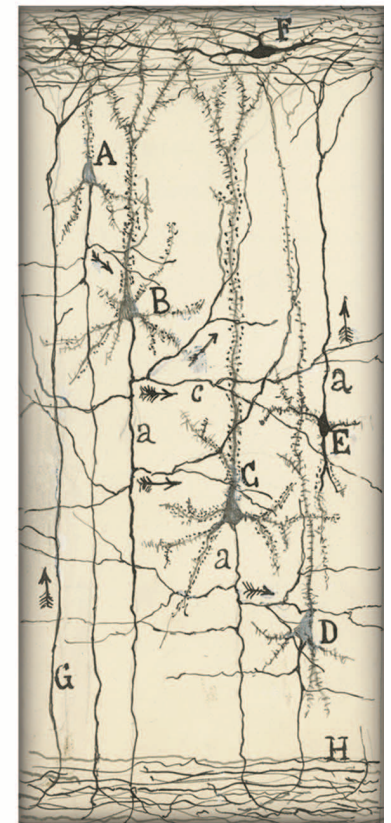
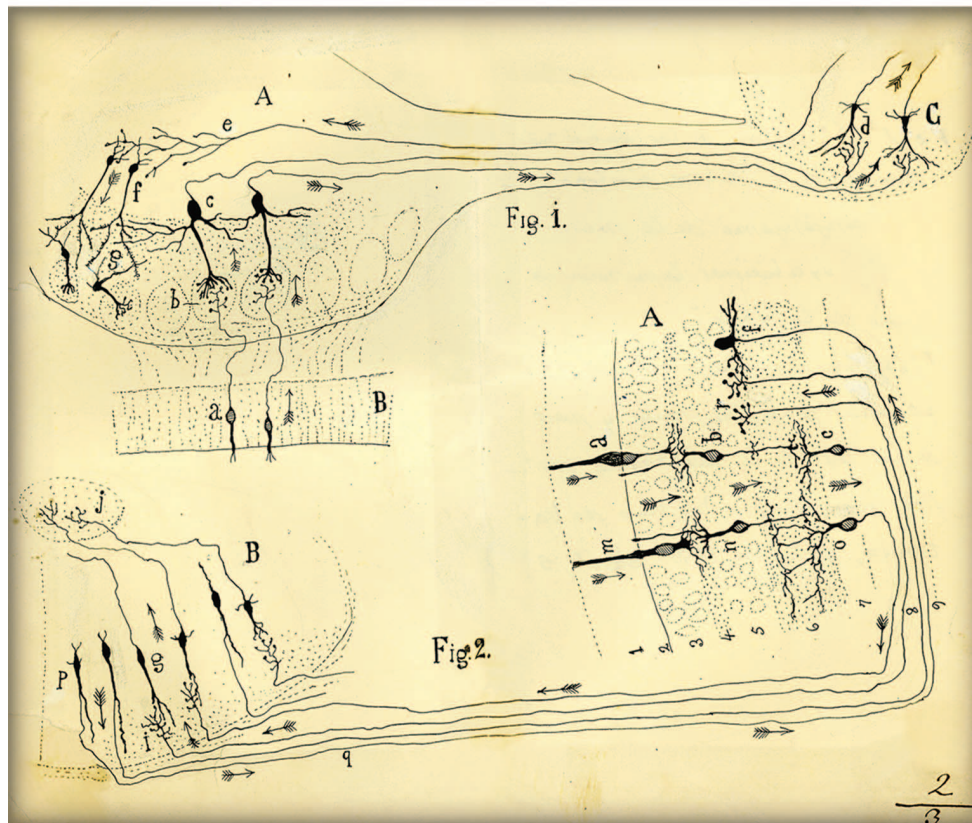


Fig. 3. (Left) Cajal's scheme showing the flow of current (arrows) in the visual and olfactory systems to support the Law of Dynamic Polarization. **(Right)** Schematic drawing by Cajal to show synaptic connections and the

possible flow of information through neural circuits in the cerebral cortex. (See supporting online material for more information.) [Copyright Herederos de Santiago Ramón y Cajal]

be studied using electron microscopy (EM) and serves to map true synaptic contacts. Thus, we propose using the terms “connectome” to refer to the map of connections at the macroscopic and intermediate levels and “synaptome” for the map at the ultrastructural level. Only by combining studies at all three levels can we fully understand the structural plan of the brain as a whole.

At present, there are powerful methods to trace the connectome in macro- (long distance connections) and microcircuits (intrinsic or local connections). These methods include classical tracing methods, as well as molecular/genetic/physiological approaches and imaging techniques, such as two-photon imaging and ontogenetic techniques (13–15). Among the most sophisticated technologies are the genetically modified mice that express fluorescent proteins in subsets of cells [e.g., the Brainbow mouse (11)]. In these animals, multicolored labeling of the axons and dendrites of particular neurons may be useful to define large-scale principles of connectivity (fig. S1).

However, it is important to bear in mind that connectivity at the light microscope level is in general rather coarse (e.g., connections between brain regions), and, in most cases, point-to-point connections between local neurons and between neurons or afferent fibers cannot be accurately determined. Indeed, axonal boutons are embedded in a complex neuropil adjacent to several possible synaptic targets. In addition, not all axonal boutons establish synaptic contacts, and a given bouton may establish several synapses (16). Thus, the presence of a labeled terminal in close apposition with a given neuronal element can only be considered as a putative synaptic contact.

To define the synaptome, serial reconstruction is the method of choice. Serial sectioning TEM is a well-established technology to obtain three-dimensional (3D) data from ultrathin sections, and it is based on imaging ribbons of consecutive sections by TEM. However, obtaining long series of ultrathin sections is extremely time-consuming and difficult, often making it impossible to reconstruct large volumes of tissue. Indeed, serial-section TEM is prone to suffer important problems, including the loss of sections, uneven section thickness, the frequent presence of debris or artifacts in the sections (e.g., folds), and geometrical distortion. To resolve these problems requires labor-intensive human interaction that prevents this approach from being widely employed. However, the recent development of automated EM techniques (17, 18) will represent an important advance in the study of the synaptome. For example, a new 3D reconstruction method involving the combination of focused ion beam milling and scanning electron microscopy (FIB-SEM) makes it possible to obtain images with a quality and resolution similar to those obtained with TEM, but with the advantage that FIB-SEM permits the rapid and automatic serial reconstruction of large tissue volumes (18). In these bricks of serial sections, the actual number of synapses per volume, their type, and their spatial distribution

can be easily defined (fig. S2). Moreover, hundreds of serial images can be obtained in a single day, whereas similar long series of sections are only occasionally obtained with most EM techniques, and it may take months or even years. The possibility of studying large volumes of tissue at the ultrastructural level, combined with various techniques (e.g., intracellular labeling of neurons, green fluorescent protein labeling of neurons, and immunocytochemistry) for correlative light and FIB-SEM microscopy, will represent a true revolution in the examination of synaptic circuits, defining the map of local and long-distance connections between the various cell types.

Nevertheless, even using this FIB-SEM technology, full reconstruction of whole brains will only be possible in some invertebrates or for relatively simple nervous systems. Indeed, even for a small mammal like the mouse, it is impossible to fully reconstruct the brain at the ultrastructural level because the magnification needed to visualize synapses yields relatively small images

and 4.5 mm in humans. Another important drawback is that the principles of anatomical design (e.g., spatial distribution, number and type of neurons, and synapses per volume) in different parts of the nervous system differ considerably, both between themselves and between species. Therefore, the data obtained in one structure cannot necessarily be applied to another, and, thus, anatomical design must be examined separately in particular regions and species.

Is the Future of the Study of Circuits So Bleak That Only Imprecise Connectomes and Incomplete Synaptomes Can Be Created?

Despite the technical difficulties, by adopting appropriate strategies with the tools now available coupled with the beginning of huge international projects like the Human Connectome Project (10) or the Blue Brain Project (19), it should be possible to make spectacular advances in unraveling brain organization, even in humans. For example, the vast majority of synapses in the

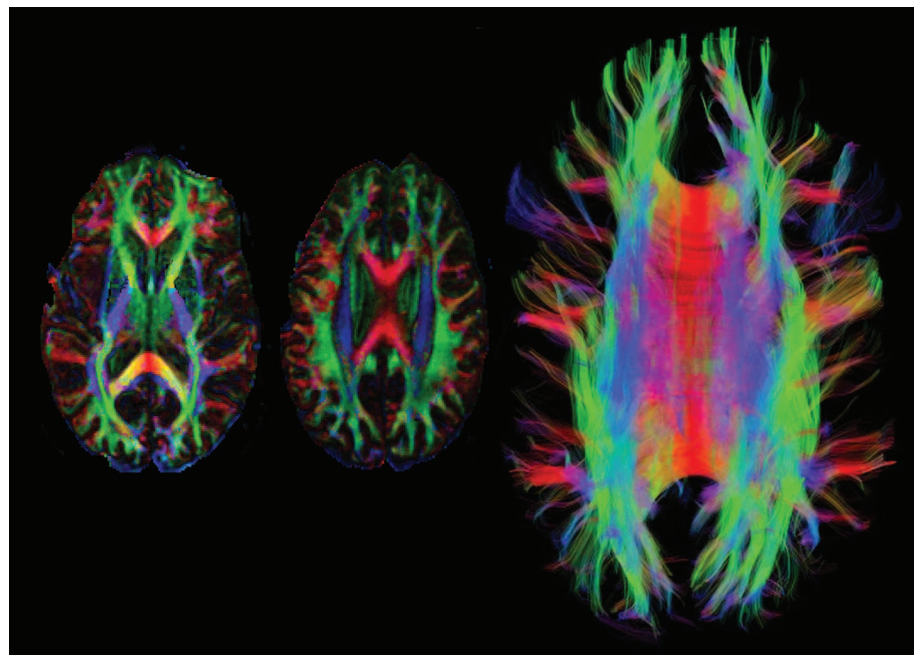


Fig. 4. Human brain connectivity. Diffusion tensor imaging of the human brain obtained from 3-tesla MRI sequences. The technique allows the direction of the tracts of white matter fibers to be visualized. (Left and Middle) Panoramic views at different levels. (Right) High-magnification tractography of the corpus callosum and superior longitudinal fasciculus. The colors indicate the direction of the fibers (red, transversal; green, anteroposterior; blue, superoinferior). [Courtesy of Juan Álvarez-Linera (Fundación CIEN-Fundación Reina Sofía, Madrid)].

(on the order of tens of μm^2). For example, it has been estimated that if we were to use sections of about $35 \mu\text{m}^2$ at a thickness of 20 nm, we would need $>1.4 \times 10^9$ sections to fully reconstruct just 1 mm^3 of tissue (18). Therefore, complete reconstructions of a small region of the mammalian brain (on the μm scale) are feasible, whereas structures like the cerebral cortex cannot be fully reconstructed because they may have a surface area of 2200 cm^2 and a thickness between 1.5

cerebral cortex are axodendritic, and, therefore, the synaptic density together with the volume fraction occupied by the neuropil will be critical factors that influence connectivity. In the human temporal cortex, the volume fraction occupied by the neuropil varies between 90 and 98%, depending on the layer (20). Nevertheless, although the synaptic density within a given area and layer may vary, this variability remains within a relatively narrow window. For instance, in the hu-

man temporal cortex and rat somatosensory cortex, the number of synaptic profiles per 100 μm^2 of neuropil varies between 20 and 36, and between 32 and 46, respectively (21). Similarly, it is expected that the variation in other ultrastructural characteristics will also fall within narrow windows, or at least that the statistical distribution of the variation can be modeled. Therefore, we will not need to reconstruct the whole layer within a given area to determine the absolute number and types of synapses, but rather the range of variability can be ascertained by multiple sampling of relatively small regions within that area. Combining these data with those obtained by light microscopy, such as the thickness of the gray matter, the volume fraction of cortical elements (neuropil, neurons, glia, and blood vessels), the density of neurons and glia per volume, the microanatomy of neurons (i.e., patterns of dendritic arbors, distribution and density of dendritic spines, and dendritic length), together with the patterns of intrinsic (intralaminar and translaminar) and long-range (corticocortical, thalamocortical, corticothalamic, and subcortical extrathalamic) connections, would seem to be the best and most feasible strategy to unravel the complex organization of the brain. For example, to determine the synaptic contribution of pyramidal cells in a given cortical layer, it is impractical to try to reconstruct all these cells at the electron microscope level. However, this parameter could be inferred by combining quantitative light microscopy data on the total number and microanatomical characteristics of these cells on the one hand, with the average density of axospinous and axodendritic synapses obtained by FIB-SEM microscopy on the other. Another approach might be to identify the nature of the axon terminals and their spatial distribution. In other words, the most appropriate route to follow now appears to be to link detailed anatomical structural data with the incomplete light and EM wiring diagrams, thereby generating a realistic statistical model, rather than attempting to fully reconstruct the nervous system.

An Additional Critical Problem: We Don't Just Have a Large Mouse Brain

The ultimate aim of neuroscience is to understand the human brain, but it is obvious that most of our present knowledge has been obtained from experimental animals. Thus, the question remains as to how much of this information can be extrapolated to humans. Although many structural aspects of the nervous system are phylogenetically conserved, others are unique to humans (21). Therefore, we have to face the additional ethical problems associated with the application of certain anatomical techniques to human material. As a result, there are considerable voids in the information available that is directly related to the microanatomy of the human brain.

However, recent technical advances coupled with the encouragement of researchers to use the human tissue available will lead to major advances in the near future. First, at the macroscopic level, techniques such as structural and functional MRI, diffusion tensor imaging, magnetoencephalography, and electroencephalography represent powerful methods to trace brain connections (Fig. 4). These techniques also serve to define general wiring principles in the brain (structural and functional), with obvious direct clinical implications (12, 22). Because these methodologies are still under continuous development, it is likely that we will soon produce even more human brain connectomes.

Light microscopy methods can now be applied to human tissue (biopsy or autopsy) that offer images of extraordinary quality. This is the case for intracellular injection in fixed material using markers like lucifer yellow. The morphology of these neurons can be visualized in such detail that they allow fine morphological details of labeled neurons to be visualized (e.g., dendritic spines) and 3D reconstructions of the dendritic arbors to be performed. In combination with immunocytochemistry for a variety of neurotransmitters, this technique can be applied to generate 3D maps of neurotransmitter-labeled appositions with the dendritic arbor of injected neurons (23) (fig. S3).

At the ultrastructural level, the analysis of specimens removed during the course of neurosurgery in patients with tumors or intractable epilepsy represents an excellent opportunity to study human brain. This is partly because the resected tissue can be immediately immersed in the fixative so that postmortem factors are mainly eliminated. Undoubtedly, this is why the quality of the EM images of this human biopsy material is comparable to that obtained in experimental animals (20). Furthermore, it is inevitable that surgical excisions pass through normal cortical regions, and, hence, this material can be exploited to analyze various ultrastructural aspects of the neocortex in detail. In this way, we can better understand the microorganization of the human brain, which would otherwise be virtually impossible to define.

The "Siliconcortex": A Fitting End to Cajal's Epic Love Story?

Finally, the cerebral cortex is the area chosen by numerous theorists and experimentalists for study because it is directly involved in many aspects of mammalian behavior. Moreover, it is the largest (85% of the brain) and most human part of the nervous system (i.e., it is the brain structure whose activity is directly related to the emergence of those capacities that distinguish humans from other mammals). During mammalian evolution, many aspects of cortical organization are maintained, and we now have the technology necessary to

examine all aspects of cortical circuitry. Indeed, this technology is currently being used by a large number of laboratories. Computational models of neuronal networks based on real circuits have become useful tools to study aspects of the functional organization of the brain (24, 25). Thus, why shouldn't it be possible to construct a "siliconcortex," a computer with an artificial cerebral cortex based on a realistic model of the complete anatomical, physiological, and molecular design of the cortical circuit? Would this not be a fitting end to the Cajalian love story?

References and Notes

1. S. R. Cajal, *Rev. Trim. Histol. Norm. Patol.* **1**, 1 (1888).
2. G. W. Bartelmez, N. L. Hoerr, *J. Comp. Neurol.* **57**, 401 (1933).
3. D. Bodian, *J. Comp. Neurol.* **73**, 323 (1940).
4. J. D. Robertson, *Proc. Soc. Exp. Biol. Med.* **82**, 219 (1953).
5. G. E. Palade, S. L. Palay, *Anat. Rec.* **118**, 335 (1954).
6. M. V. Bennett, *Brain Res. Brain Res. Rev.* **32**, 16 (2000).
7. K. Fuxe *et al.*, *Brain Res. Brain Res. Rev.* **55**, 17 (2007).
8. G. Perea, A. Araque, *Brain Res. Brain Res. Rev.* **63**, 93 (2010).
9. G. M. Shepherd, in *Encyclopedia of Neuroscience*, G. Adelman, B. H. Smith, Eds. (Elsevier, New York, 2004).
10. O. Sporns, G. Tononi, R. Kötter, *PLOS Comput. Biol.* **1**, e42 (2005).
11. J. W. Lichtman, J. Livet, J. R. Sanes, *Nat. Rev. Neurosci.* **9**, 417 (2008).
12. B. Biswal *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **107**, 4734 (2010).
13. E. G. Jones, *Brain Res. Brain Res. Rev.* **55**, 248 (2007).
14. V. Nikolenko *et al.*, *Front. Neural Circuits* **2**, 5 (2008).
15. A. Li *et al.*, *Science*; published online 4 November 2010 (10.1126/science.1191776).
16. L. Descarries, N. Mechawar, *Prog. Brain Res.* **125**, 27 (2000).
17. K. L. Briggman, W. Denk, *Curr. Opin. Neurobiol.* **16**, 562 (2006).
18. A. Merchán-Pérez, J. R. Rodríguez, L. Alonso-Nanclares, A. Schertel, J. DeFelipe, *Front. Neuroanat.* **3**, 18 (2009).
19. H. Markram, *Nat. Rev. Neurosci.* **7**, 153 (2006).
20. L. Alonso-Nanclares, J. Gonzalez-Soriano, J. R. Rodríguez, J. DeFelipe, *Proc. Natl. Acad. Sci. U.S.A.* **105**, 14615 (2008).
21. J. DeFelipe, L. Alonso-Nanclares, J. I. Arellano, *J. Neurocytol.* **31**, 299 (2002).
22. A. W. Toga, P. M. Thompson, S. Mori, K. Amunts, K. Zilles, *Nat. Rev. Neurosci.* **7**, 952 (2006).
23. R. Benavides-Piccione, J. I. Arellano, J. DeFelipe, *Cereb. Cortex* **15**, 1584 (2005).
24. C. Koch, I. Segev, *Nat. Neurosci.* **3** (suppl.), 1171 (2000).
25. T. Binzegger, R. J. Douglas, K. A. Martin, *Neural Netw.* **22**, 1071 (2009).
26. J. DeFelipe, *Cajal's Butterflies of the Soul: Science and Art* (Oxford Univ. Press, New York, 2010).
27. S. Ramón y Cajal, *Recuerdos de mi Vida* (Moya, Madrid, 1917).
28. This work has been supported by the Spanish Ministry of Science and Innovation (Cajal Blue Brain Project).

Supporting Online Material

www.sciencemag.org/cgi/content/full/330/6008/1198/DC1
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SOM Text

Figs. S1 to S3

References

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Crystal Structure of the Eukaryotic Ribosome

Adam Ben-Shem,* Lasse Jenner, Gulnara Yusupova, Marat Yusupov*

Crystal structures of prokaryotic ribosomes have described in detail the universally conserved core of the translation mechanism. However, many facets of the translation process in eukaryotes are not shared with prokaryotes. The crystal structure of the yeast 80S ribosome determined at 4.15 angstrom resolution reveals the higher complexity of eukaryotic ribosomes, which are 40% larger than their bacterial counterparts. Our model shows how eukaryote-specific elements considerably expand the network of interactions within the ribosome and provides insights into eukaryote-specific features of protein synthesis. Our crystals capture the ribosome in the ratcheted state, which is essential for translocation of mRNA and transfer RNA (tRNA), and in which the small ribosomal subunit has rotated with respect to the large subunit. We describe the conformational changes in both ribosomal subunits that are involved in ratcheting and their implications in coordination between the two associated subunits and in mRNA and tRNA translocation.

The ribosome is responsible for translating the information encoded by mRNA into protein in all living cells. It is composed of two subunits that consist of multiple proteins and RNA. The structures of the prokaryotic ribosome and individual subunits reveal in fine detail the evolutionarily conserved catalytic core where peptide bonds are formed and correct aminoacyl-transfer RNA (tRNAs) are selected to complement mRNA codons (1–11). Other activities of the ribosome that also constitute integral steps in the translational process do not show such a high degree of conservation. In particular, prokaryotes and eukaryotes use fundamentally different mechanisms for initiation of protein synthesis (12–14). The eukaryotic cap-dependent initiation is a highly regulated process that entails scanning of the mRNA from the 5'-cap moiety downstream to the start codon. Many viruses (e.g., hepatitis C virus) repress cap-dependant translation and utilize special sequences in their mRNA, known as internal ribosome entry sites (IRESs), that can directly recruit the ribosome to the viral start codon and thus hijack the translational machinery (14).

The eukaryotic ribosome has a sedimentation coefficient of 80S and, with a minimal mass of ~3.3 MD (yeast and plants), is roughly 40% larger than its bacterial counterpart (70S). The large 60S subunit consists of three rRNA (rRNA) molecules (25S, 5.8S, and 5S) and 46 proteins, whereas the small 40S subunit includes only one rRNA chain (18S) and harbors 33 proteins. Several components contribute to the greater weight and complexity of eukaryotic ribosomes: RNA expansion segments that are inserted into the evolutionarily conserved rRNA core, 25 additional proteins

with no homologs in eubacteria, as well as extra protein segments, mainly in the N' or C' tails of the conserved proteins. The precise functional roles of these additional components are largely unknown. Cryo-electron microscopy (cryo-EM) provided the first models of eukaryotic ribosomes at resolutions between 6.1 and 15 Å, which revealed the locations and shapes of the RNA expansion segments and indicated the position of additional protein moieties (15–17).

The higher complexity and larger size of the eukaryotic ribosome is amply reflected in the vast number of proteins that interact with it and the variety of cellular processes in which the ribosome or its subunits play key roles. Examples are nonsense-mediated decay, unfolded protein response, and various eukaryote-specific translation regulation mechanisms.

We crystallized the complete eukaryotic 80S ribosome from *Saccharomyces cerevisiae* and determined its structure at 4.15 Å resolution. Our crystals capture the ribosome in the so-called ratcheted state. It was postulated more than 40 years ago that the translocation of mRNA and tRNA during protein synthesis is coupled to intersubunit movements (18, 19). More recent cryo-EM studies suggested that translocation is facilitated by large-scale movements involving a rotation of the small subunit relative to the big subunit (20). As confirmed by various studies, this ratchetlike intersubunit reorganization of the ribosome is essential for translocation (21). Recently, structures of the *Escherichia coli* ribosome trapped in intermediate states of ratcheting were described (22); however, an x-ray structure of the ribosome in the fully ratcheted state was lacking.

Structure determination. The dynamic nature of the eukaryotic ribosome has hindered efforts to crystallize it. To overcome this obstacle, we used the fact that depletion of glucose from yeast growth medium for a short time results in inhibition of translation initiation and accumulation of a homogeneous population of vacant ribosomes (23, 24). We

then purified them in a way that minimized damage to ribosomes during and after cell lysis (24).

Initial crystals diffracted poorly and were improved by soaking with various dehydration agents and metal ions. These improvements yielded three crystal forms—form I with four ribosomes in the asymmetric unit and forms II and III with two—all belonging to space group $P2_1$ but differing in their cell parameters. Form III diffracts to better than 3 Å resolution, but in order to obtain a complete data set from a single crystal—to take full advantage of the anomalous signal from the bound osmium ions—we collected a data set at 4.15 Å resolution, with reflections useful for refinement to 4 Å (table S1).

Molecular replacement (MR) procedures found clear solutions in all crystal forms when a search model was used that was composed of the large subunit (50S) from *Haloarcula marismortui* (8) and the small subunit (30S) from *Thermus thermophilus* (1). This solution, after improvement by rigid-body refinement, was used to locate several hundred osmium sites in form III and thus obtain initial single-wavelength anomalous dispersion (SAD) phases. However, maps from the combined MR + SAD phases were not readily interpretable. Therefore, iterative rounds of the following scheme were performed: (i) phase improvement by using solvent flattening, and inter-crystal and noncrystallographic symmetry averaging between the three forms; (ii) expansion of the model by manual building, followed by rigid-body refinement of large domains; and (iii) location of additional osmium sites by using the expanded model and recalculation of maps. When the model approached completion, the number of located osmium sites had reached 700. At that point, averaging was no longer used, and solvent-flattened SAD or model + SAD phases guided the final building steps.

The model consists of two ribosomes in the asymmetric unit of crystal form III and contains the entire rRNA moiety except for ES27 and a small part of ES7, both in 60S, as well as a few residues in ES6 of 40S. Density for the phosphates was usually clear and guided modeling of the rRNA parts. The model also contains the Ca backbone of all proteins with homologs in prokaryotic ribosome x-ray structures, including, in most cases, their eukaryote-specific additions. Three additional proteins whose location was identified by cryo-EM (L30e, S19, and RACK1) (25–28) were also resolved. The model includes many additional α helices and β strands that belong to eukaryote-specific proteins. With the exception of several proteins (S25, S17, S28, L14, and L6), we refrained from assigning these secondary-structure elements to individual proteins because biochemical data that may confirm their position are lacking. The model also contains 715 osmium hexamine molecules that probably represent sites of magnesium ions.

This paper will focus on only one of the two ribosomes in the asymmetric unit, which assumes the same conformation as all the monomers in the other two crystal forms and fits well into cryo-EM maps of yeast ribosomes (16, 26). The second

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monomer, whose small subunit has a somewhat different conformation, may represent some intermediate state during translocation. The refinement of this monomer is ongoing, and it will not be further described here.

We use the common notation for ribosomal components, where ES x denotes expansion segment number x ; h x and H x refer to helices within the conserved core of the small and large subunit rRNA, respectively; and L x and S x are large subunit and small subunit proteins.

Overall view of the 80S ribosome: An elaborate network of interactions. The overall structure of the eukaryotic ribosome reveals a considerably larger assembly than its prokaryotic counterpart, but the basic architecture remains similar with common recognizable landmarks (Fig. 1, A and B). The rRNA expansion elements are located predominantly on the solvent-exposed sides at the periphery of both subunits. All inter-subunit bridges described in the crystal structure of the bacterial ribosome (5) have corresponding bridges in the yeast ribosome (Fig. 1C, and table S2). In addition, there are several eukaryote-specific bridges that were first visualized by low-resolution cryo-EM studies of yeast ribosomes (15, 16) and whose components are more accurately described now by our 4.15 Å model (Fig. 1C and table S2). The quality of the electron density is shown in Fig. 1, D and E.

The eukaryote-specific elements substantially expand the network of interaction within the ribosome. Perhaps the most impressive example is the ~200-nucleotide-long ES6 of the small subunit (Fig. 2). This expansion segment emerges at the solvent face of the platform, just below helix 26 (h26), where it is enveloped by several eukaryote-specific protein moieties, including the 60-residue-long α -helical extension at the C terminus of protein L19e, thus forming a eukaryote-specific bridge (eB12) (Fig. 2). ES6 then sends one of its three long arms in the direction of the shoulder (Fig. 2). This arm interacts with protein S22 (S8p); its tip branches into two loops, one of which lies in close proximity to a eukaryote-specific helix-turn-helix insertion in protein S9 (S4p). The nearness of ES6 to h26 and S9, components of the exit and entry sites of the mRNA, respectively, suggests its involvement in translation initiation, perhaps as a docking surface for factors that participate in activities at both sites (29). The second long arm of ES6 might provide additional binding surface for some interacting partners of the ribosomes. The third long arm of this expansion segment runs down toward the bottom of the small subunit, where its tip is enveloped by the two internal loops of ES3. A eukaryote-specific protein that we could partially model is bound to ES3 and h9 and forms a eukaryote-specific bridge (eB11) with ES41 of 60S. Thus, at the bottom of the 40S body, several expansion segments and proteins create a eukaryote-specific network of interactions.

Ribosomal domain movements in the ratcheted state. Our model of the eukaryotic ribosome, when compared with the structure of the

nonratcheted prokaryotic ribosome (1), shows a 4° to 5° counterclockwise rotation of 40S with respect to 60S and swiveling of the head domain of 40S by 15.5° in the direction of the tRNA exit site (Fig. 3, A and B). These are the characteristics of the ratcheted state, consistent with observations made by cryo-EM studies, that the yeast ribosome devoid of ligands assumes the same ratcheted conformation as the one stabilized by the binding of eukaryotic elongation factor 2 (16).

In comparison with nonratcheted prokaryotic ribosomes, the large-scale movements implicated in ratcheting result in substantial alterations in the bridges between the head domain of 40S and the large subunit (1). The first bridge between the head domain and the large subunit, bridge B1a (Fig. 1C), is formed in the nonratcheted 70S by

the A-site finger (H38 of 23S) and protein S13p. Because swiveling displaces components at the periphery of the head domain by as much as 25 Å, this bridge is rearranged in our model. We find that ratcheting brings residues 1239 to 1241 at the tip of h33 (component of the beak of 40S), as well as protein S15 (S19p), into the vicinity of the tip of H38, which bends considerably in order to form interactions with these partners (Fig. 3, C and D). Conformational changes are also observed at the base of H38 where it contacts the central protuberance. In the second bridge between the head domain of 40S and the large subunit, B1b (Fig. 1C), the large shift in the position of protein S18 (S13p) places its longest helix, instead of the N-terminal loop, in contact with central protuberance protein L11 (L5p) (Fig.

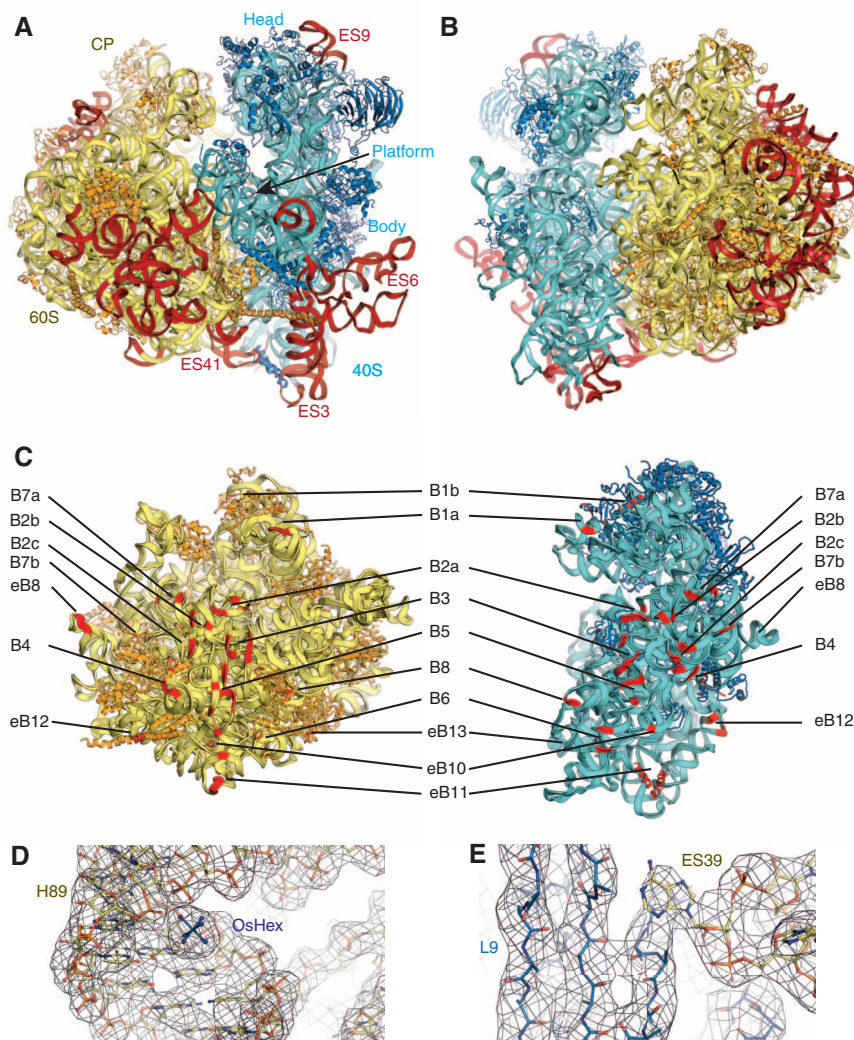


Fig. 1. Overall view of the x-ray structure of *S. cerevisiae* 80S ribosome. (A) View from the E site. Proteins and rRNA in the 40S are colored dark and light blue, respectively, and dark and pale yellow, respectively, in the 60S (in all the following figures, this color scheme will be maintained unless otherwise indicated). Expansion segments are in red. (B) View from the A site. (C) Interface views of the 60S and 40S subunits with bridges numbered essentially as in (5), and colored red. (D and E) Electron density maps, calculated with SAD + model combined phases and contoured at 1.5 σ , showing (D) H89 of 25S with an osmium hexamine molecule bound in the major groove of H89 and (E) interaction between ES39 and protein L9. This and all other figures were made with Pymol (43).

3, C and E). Similar rearrangements were observed in structures of partially ratcheted *E. coli* 70S ribosomes (22). In addition, residues 70 to 75 from a loop in S15 (S19p) may also interact with L11 (L5p) in the ratcheted state. The prokaryotic homologs of the two proteins that are strongly shifted, S15 (S19p) and S18 (S13p), were shown in the nonratcheted state to monitor the occupancy of the A and P sites, respectively (Fig. 3F) (1, 4, 30). The direct interaction between these two proteins is probably stronger in eukaryotes because of additional residues in both.

The entire central protuberance of the large subunit, dominated by 5S rRNA and the proteins enveloping it, undergoes considerable structural rearrangement. These alterations involve tilting of 5S helices, a shift in the position of L11 (L5p) elements, and displacement by up to 7 Å of all L5 (L18p) domains except the N tail and the first helix (Fig. 3, G to I). Their plasticity in response to ratcheting and their strategic position in the large subunit suggest that the central protuberance and the A-site finger may coordinate changes in different sites of the large 60S subunit, notably the L1 protuberance, the GTPase (guanosine triphosphatase) center, and the peptidyl-transferase

center, with the 40S head rotation and with mRNA translocation. This proposed requirement for plasticity of the central protuberance might underlie the need for keeping 5S as a separate rRNA chain.

The numerous interactions between the head and the large subunit may serve to limit and to monitor the extent of the head's rotation. All these interactions are relatively weak and entail large distances but together facilitate a wide, but precise, ratcheting movement. Flexibility or plasticity of the interacting partners is likely crucial for constantly adjusting the bridges as the ratcheting movement progresses.

The central protuberance in eukaryotes is larger than its prokaryotic counterpart and forms additional interactions with other regions in the ribosome. Comparing our model with the archeal large subunit (Fig. 3, G and H) (8), we find that these additional features include ES12, which forms an RNA helix that runs parallel to 5S rRNA; the eukaryote-specific protein L6e, which binds 5S rRNA and is embedded in a region rich in other eukaryote-specific elements [protein L14e, the N-terminal domain of L7 (L30p), and ES7]; and eukaryote-specific domains in proteins L5 (L18p), L21e, and L10e (L16p).

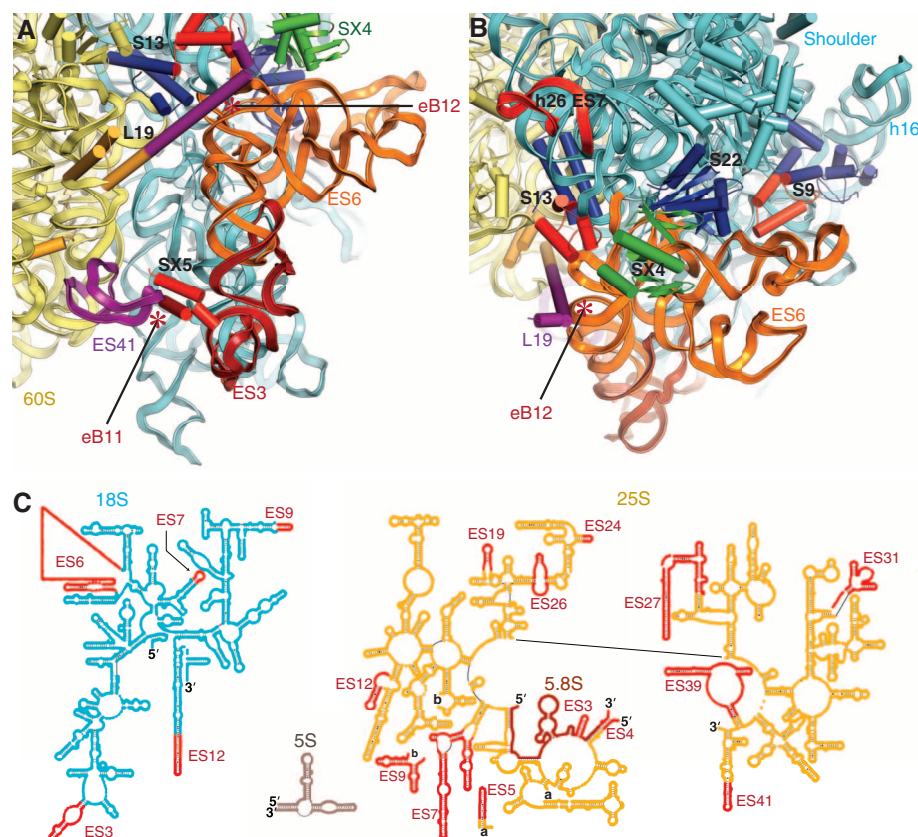


Fig. 2. Part of the extended network of interactions formed by eukaryote-specific elements. (A and B) ES6 (orange) forms bridge eB12 with the eukaryote-specific α -helical addition (colored in magenta) at the C terminus of protein L19e. Another eukaryote-specific bridge, eB11, is formed between ES41 of 25S rRNA (magenta) and a eukaryote-specific protein bound to ES3 and h9 of 40S. Bridges are marked with asterisks. (C) Secondary structure diagram of 18S rRNA in blue, 5S rRNA in brown, 25S rRNA in yellow, and 5.8S in dark red, showing expansion segments in red (Comparative RNA Web site of RNA: <http://www.rna.cccb.utexas.edu>).

Rearrangement of ribosome functional sites upon ratcheting. In structures of the prokaryotic ribosome at putative intermediate states along the ratcheting pathway (22), the central bridges are almost indistinguishable from those observed in the nonratcheted state. In contrast, we find that in our fully ratcheted state some of these bridges undergo considerable alterations. Bridge B2a is of special interest, as it is formed by the base of the penultimate stem (h44) of 18S and H69 of 25S. In this universally conserved region, the residues 1755 and 1756 (1492 and 1493 in *E. coli*) of 18S and 2256 (1913 in *E. coli*) of 25S RNA play a key role in the decoding of mRNA (10). We find that in the ratcheted yeast ribosome, the nucleotides 1755 and 1756 and the region of 18S rRNA encompassing these decoding elements undergo a local conformation change. The RNA strand carrying residues 1754 to 1758 (1491 to 1495) is twisted and pushed away from the mRNA pathway by up to 9 Å. This position would preclude interactions of the rRNA backbone with the mRNA-tRNA complex (Fig. 4A). In addition, the tip of H69 bends so as to maintain bridge B2a, and several residues involved in the bridge assume different orientation compared with the vacant nonratcheted ribosome. We suggest that the rotation of the small subunit results in breaking or at least considerably loosening of the interaction of the 40S body with the mRNA-tRNA complex, so that this complex would be free to follow the rotation of the head. These findings concur with the notion that ratcheting is a multistep process (22) and suggest that rotation of the body and rearrangement of the decoding region occur before rotation of the head.

Other instances where ratcheting breaks interactions that may impede translocation include the bending of the A-site finger, which removes its interaction with the elbow of A-tRNA, and breaking of the contact between A1001 of 18S (A790) and P-tRNA through the rotation of the 40S platform. However, the swiveling of the head alone can account only for 13 to 15 Å of movement of the mRNA-tRNA complex, out of the required 20 Å (one codon) distance. To complete the translocation step and proceed to the non-ratcheted posttranslocation state, further conformational changes that would result in breaking the bonds between the head and the mRNA-tRNA complex must ensue (1, 4, 5). Our model suggests that these changes would entail large structural rearrangement in the head domain. Considering, for example, the steric block between P-tRNA and the E site formed by the universally conserved ridge of residues 1575 to 1578 in 18S (1338 to 1341 in *E. coli*, fig. S1) (11). In our structure, this ridge has rotated with the head in a trajectory that passed through the E site, which suggests that the forward swiveling of the head maintains the position of the ridge with respect to P-tRNA (Fig. 4B and fig. S1B). Hence, the ridge creates a large physical barrier that prevents further forward movement and release of the tRNA, whereas the tRNA physically prevents back-ratcheting.

A large conformational change, driven perhaps by the energy of guanosine triphosphate hydrolysis (31), is likely required to remove this barrier. The nature and timing of events that complete the translocation of the mRNA-tRNA complex are unknown.

Adjustment of intersubunit contacts upon ratcheting. Practically no alterations were observed at bridge B3, which may constitute the center of rotation for the small subunit. Bridges B2b and B2c also show small variations com-

pared with the nonratcheted state of the prokaryotic ribosome (1). However, other bridges involving h44 undergo substantial changes and are weakened considerably because of the bending of this long rRNA helix just below bridge B3. Thus, most of the interactions that constituted bridges B5 and B6 are broken in the ratcheted state, although the bridges are still weakly maintained through a few interactions (table S2). A eukaryote-specific protein that we could not assign is bound to h44 and h8 and approaches protein L3 (L3p) to form a

eukaryote-specific bridge (eB13) (Fig. 1C and table S2). The extent of h44 bending may be smaller in prokaryotes where eB13 cannot form.

Considerable adaptation of both subunits is required to maintain the bridges that are far from the center of rotation. This is particularly true in the case of bridge B4, which connects the platform of the small subunit with H34 of the large subunit. Upon ratcheting, the tip of H34 bends considerably in order to establish interaction with residues 629 to 631 in h20 of the small subunit, as

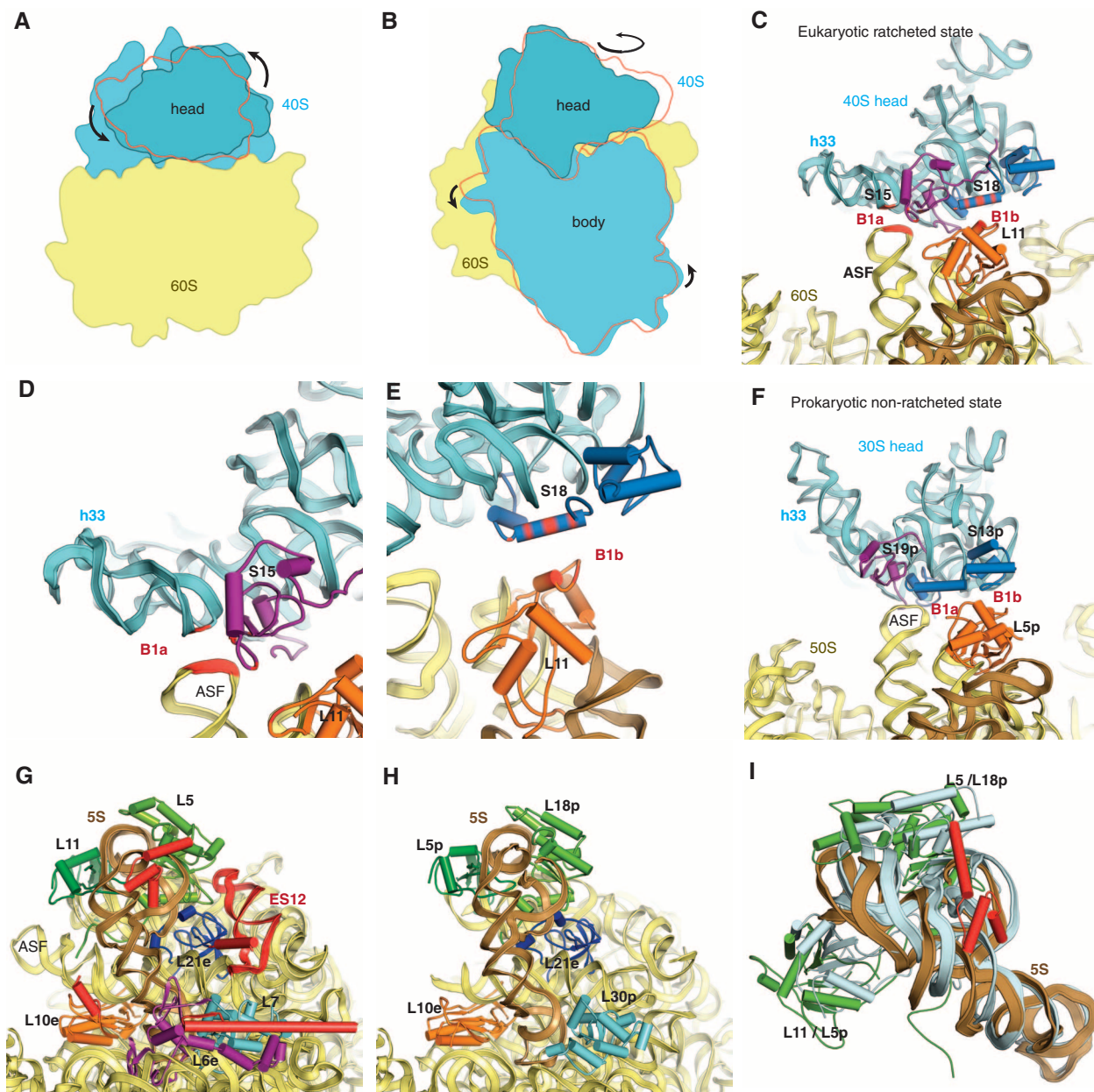


Fig. 3. Ratcheted state of the eukaryotic 80S ribosome. (A and B) Schematic representation of the motion from the nonratcheted to the ratcheted state. The red line indicates the outline of the 40S in the nonratcheted state, with arrows indicating the trajectory. (A) Top view of the yeast 80S ribosome. (B) View from the solvent side of 40S. (C) Bridge B1 in the ratcheted 80S. (D) Close-up view of the yeast B1a bridge. The tip of the A-site finger (ASF-H38) from 25S rRNA forms interactions (colored in red) with the head of the 40S subunit, including protein S15 (magenta). (E) View of the yeast bridge B1b formed between pro-

teins S18 and protein L11. Residues thought to interact are indicated in red. (F) Bridge B1 as found in the nonratcheted prokaryotic 70S ribosome. (G and H) The central protuberance (CP) of (G) *S. cerevisiae* 60S (eukaryote-specific elements marked in red) and (H) the archeal *H. marismortui* 50S. (I) The central protuberance of the 50S prokaryotic ribosome (light blue) superimposed on the eukaryotic ratcheted 60S (5S rRNA in brown, proteins in green, and eukaryote-specific elements in red). All parts except 5S rRNA and proteins L5 (L18p) and L11 (L5p) have been removed for clarity.

well as the C-terminal helix of protein S13 (S15p), which also undergoes some changes (Fig. 4, C and D). This bridge is mediated only by RNA-protein interactions in the nonratcheted prokaryotic ribosome, whereas in the ratcheted state, RNA-RNA interactions play an important role (table S2).

The L1 protuberance. Large-scale movements of the L1 protuberance, which consists of a stalk (H76) and an upper part [protein L1 (L1p) and helices H77 and H78], are associated with translocation (32). These movements are thought to play a key role in the release of E-site tRNA. In our model, the L1 protuberance reaches deep into the intersubunit space and is stabilized by interactions with protein L42e (L33p) (Fig. 5A). A similar position of the L1 protuberance was observed in cryo-EM studies of a translocation intermediate of a prokaryotic ribosome complex with tRNA in the P/E state (33). This is in stark contrast to the position of the L1 protuberance observed in

structures of nonratcheted 70S ribosomes where it extends away from the body of the large subunit (1, 11) (Fig. 5, B and C). The conformational change between the in- and outward positions likely entails tilting of the stalk around its base, as well as additional rotation and tilting of the upper part of the protuberance because of a hinge point that locally breaks the helical conformation at the middle of the stalk. At this hinge point, a eukaryote-specific bulged-out nucleotide and a series of species-dependent weak base pairs, probably render the L1 protuberance more flexible in eukaryotes (fig. S2). It is noteworthy that the largest movements of the L1 protuberance have been observed in yeast ribosomes (16). In cryo-EM studies, it was shown that the fungi-specific elongation factor (eEF3), the so called “E-site factor” (34, 35), stabilizes the outward position of the L1 protuberance and thus allows E-tRNA release. The binding site for eEF3 includes the central protuberance of 60S, where the presence

of the factor prevents the L1 protuberance from adopting the fully inward position (34). However, it is not clear how eEF3 binding stabilizes the outward position that facilitates E-site tRNA release. In view of our finding regarding the central protuberance dynamics, it is possible that eEF3 could exert its effect through inducing conformational changes in this domain of the ribosome.

Structure of the entry and exit sites of mRNA—implication to initiation. The entry and exit sites of the mRNA (36) on the small subunit reveal features that are unique to eukaryotes and may pertain to the ribosome’s interactions with mRNA and initiation factors. At the entry site in prokaryotes, h16 assumes a closed conformation, so that its tip is in proximity to S3p, a protein that forms the mRNA entry tunnel together with proteins S4p and S5p (1, 36) (Fig. 5E). In contrast, this helix bends in eukaryotes to adopt an open orientation that extends away from the body (Fig. 5F) (15). In prokaryotes, a domain that belongs to S4p, composed of two α helices, forms strong interactions and virtually covers a large part of the RNA helix h16 (1, 4). In the eukaryotic homolog of S4p, protein S9, this two-helix domain does not exist, and the C-terminal helix that could potentially interact with h16 tilts away. Thus, in yeast, h16 is bare, with no rRNA-protein interactions, and is free to rotate around its base. These observations must be viewed within the context of the eukaryotic initiation step. Current models suggest that binding of factors eIF1 and eIF1A to 40S stimulates scanning by inducing h16 to adopt a closed conformation, which stabilizes an opening of the mRNA entry tunnel latch (13, 37). Binding of IRES to the 80S ribosome induces similar changes (38, 39).

The entry tunnel latch is formed by interactions between the beak of the small subunit and h18 in the body. The beak in eukaryotes has a considerably different structure and harbors an additional protein moiety, partially modeled here as the eukaryote-specific protein S17 (fig. S3).

The exit site of the mRNA is more intricate in eukaryotes than in prokaryotes and contains several additional components (Fig. 5G). In association with protein S5 (S7p), just above the mRNA path, a eukaryote-specific protein with a high β -strand content is poised to bind mRNA. We modeled this protein as S28e in accordance with biochemical data (40). We also located several protein secondary structure elements situated just below the proposed mRNA path, in a position similar to that occupied in prokaryotes by protein S18p, which binds the Shine-Dalgarno sequence (3, 41, 42). These helices and strands probably belong to protein S26e and one additional unidentified protein (indicated together in Fig. 5G as protein SX2). A eukaryote-specific bridge eB8 is likely formed between this protein moiety and the first of two arms of ES31 in 25S (Fig. 5G). In addition, ES7 of 18S, an extension of h26, forms part of the mRNA exit site.

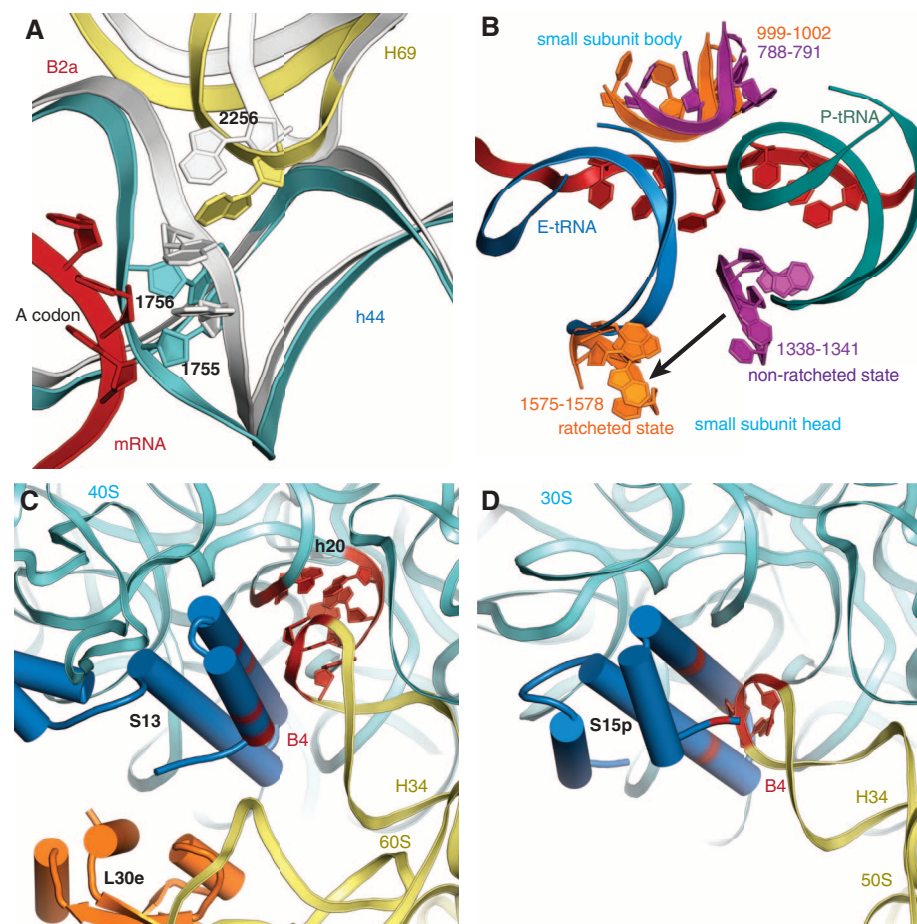


Fig. 4. Rearrangement of ribosome functional sites upon ratcheting. (A) Superposition of the prokaryotic nonratcheted ribosome on the ratcheted yeast ribosome. View of the decoding region with prokaryotic 16S and 23S rRNA in gray, the A codon of mRNA in red, and eukaryotic 18S and 25S RNA in blue and yellow, respectively. (B) Top view of the superposition of the 70S ribosome containing P-tRNA (green), E-tRNA (blue), and mRNA (red) on the 80S ratcheted ribosome. The ridge consisting of nucleotides 1575 to 1578 (1338 to 1341 in prokaryotes) forming the steric block between P-tRNA and E-tRNA and the region 999 to 1002 (788 to 791 in prokaryotes) from the platform of the small subunit. (C and D) View of the intersubunit bridge B4 in yeast (C) and in prokaryotes (D). Intersubunit contacts are colored in red.

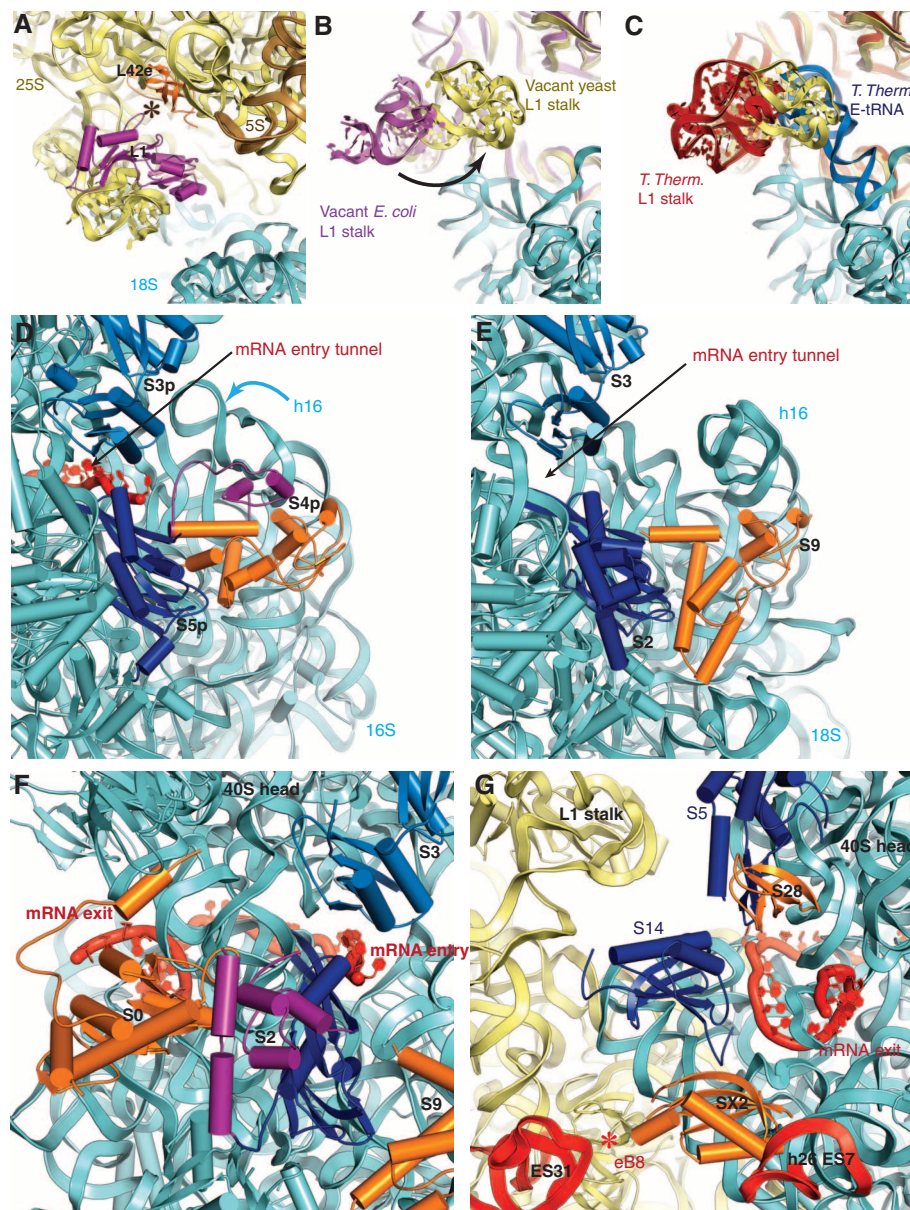


Fig. 5. The L1 protuberance and mRNA entry and exit sites. **(A)** Top view of the L1 protuberance in the yeast ratcheted ribosome. The interaction between protein L1 (magenta) and protein L42e (L33p) (orange) is marked with an asterisk. **(B)** Top view of the superposition of the nonratcheted vacant prokaryotic ribosome (23S shown in magenta) on the vacant eukaryotic ribosome (25S yellow, 18S blue), showing the movement of the L1 protuberance. Protein L1 has been removed for clarity. **(C)** Superposition showing the L1 protuberances of the yeast ribosome and the prokaryotic ribosome with bound E-tRNA (23S shown in red and E-tRNA in blue). **(D)** and **(E)** Solvent side view of the mRNA entry site on the small subunit in the (D) prokaryotic (mRNA in red) and in the (E) yeast ribosome. The additional prokaryotic domain of S4p is shown in magenta. **(F)** View of the back of the 40S, showing the connection between the mRNA exit and entry sites in the yeast ribosome (mRNA from the prokaryotic model in red). The eukaryote-specific additions to the N and C termini of S2 (S5p) are shown in magenta. **(G)** The eukaryotic mRNA exit site (mRNA from the prokaryotic model in red). The eukaryote-specific bridge eB8 is marked with an asterisk.

Another characteristic of the eukaryotic ribosome in this region is a direct contact between the mRNA entry and exit sites, which is established by a strong interaction between S0 (S2p), a part of the mRNA exit site, and eukaryote-specific extensions to protein S2 (S5p), a component of the mRNA entry tunnel (Fig. 5F).

Conclusion. The crystal structure of the complete 80S eukaryotic ribosome presented here allows rationalization, in structural terms, of existing biochemical and genetic information and will facilitate the design of future experimental models for investigating various aspects of protein synthesis. Further high-resolution structures of the eukaryotic

ribosome—from yeast (as well as other species of eukaryotes) with its plethora of substrates, factors, and protein partners—coupled with biochemical and biophysical studies will be needed to provide a molecular description of such complex phenomena as translation initiation, regulation, and ribosome assembly, as well as for the development of drugs that will target the translational machinery.

References and Notes

1. L. B. Jenner, N. Demeshkina, G. Yusupova, M. Yusupov, *Nat. Struct. Mol. Biol.* **17**, 555 (2010).
2. M. Laurberg *et al.*, *Nature* **454**, 852 (2008).
3. G. Yusupova, L. Jenner, B. Rees, D. Moras, M. Yusupov, *Nature* **444**, 391 (2006).
4. M. Selmer *et al.*, *Science* **313**, 1935 (2006).
5. M. M. Yusupov *et al.*, *Science* **292**, 883 (2001).
6. J. Harms *et al.*, *Cell* **107**, 679 (2001).
7. P. Nissen, J. Hansen, N. Ban, P. B. Moore, T. A. Steitz, *Science* **289**, 920 (2000).
8. N. Ban, P. Nissen, J. Hansen, P. B. Moore, T. A. Steitz, *Science* **289**, 905 (2000).
9. B. T. Wimberly *et al.*, *Nature* **407**, 327 (2000).
10. J. M. Ogle *et al.*, *Science* **292**, 897 (2001).
11. B. S. Schuwirth *et al.*, *Science* **310**, 827 (2005).
12. R. J. Jackson, *Biochem. Soc. Trans.* **33**, 1231 (2005).
13. R. J. Jackson, C. U. Hellen, T. V. Pestova, *Nat. Rev. Mol. Cell Biol.* **11**, 113 (2010).
14. N. Sonenberg, A. G. Hinnebusch, *Cell* **136**, 731 (2009).
15. C. M. Spahn *et al.*, *Cell* **107**, 373 (2001).
16. C. M. Spahn *et al.*, *EMBO J.* **23**, 1008 (2004).
17. T. Becker *et al.*, *Science* **326**, 1369 (2009).
18. M. S. Bretscher, *Nature* **218**, 675 (1968).
19. A. S. Spirin, *Cold Spring Harb. Symp. Quant. Biol.* **34**, 197 (1969).
20. J. Frank, R. K. Agrawal, *Nature* **406**, 318 (2000).
21. L. H. Horan, H. F. Noller, *Proc. Natl. Acad. Sci. U.S.A.* **104**, 4881 (2007).
22. W. Zhang, J. A. Dunkle, J. H. Cate, *Science* **325**, 1014 (2009).
23. M. P. Ashe, S. K. De Long, A. B. Sachs, *Mol. Biol. Cell* **11**, 833 (2000).
24. Materials and methods are available as supporting material on Science Online.
25. M. Halic, T. Becker, J. Frank, C. M. Spahn, R. Beckmann, *Nat. Struct. Mol. Biol.* **12**, 467 (2005).
26. D. J. Taylor *et al.*, *Structure* **17**, 1591 (2009).
27. J. Sengupta *et al.*, *Nat. Struct. Mol. Biol.* **11**, 957 (2004).
28. Single-letter abbreviations for the amino acid residues are as follows: A, Ala; C, Cys; D, Asp; E, Glu; F, Phe; G, Gly; H, His; I, Ile; K, Lys; L, Leu; M, Met; N, Asn; P, Pro; Q, Gln; R, Arg; S, Ser; T, Thr; V, Val; W, Trp; and Y, Tyr.
29. A. Marintchev *et al.*, *Cell* **136**, 447 (2009).
30. L. Jenner, N. Demeshkina, G. Yusupova, M. Yusupov, *Nat. Struct. Mol. Biol.* **17**, 1072 (2010).
31. M. V. Rodnina, A. Savelsbergh, V. I. Katunin, W. Wintermeyer, *Nature* **385**, 37 (1997).
32. A. Korostelev, D. N. Ermolenko, H. F. Noller, *Curr. Opin. Chem. Biol.* **12**, 674 (2008).
33. M. Valle *et al.*, *Cell* **114**, 123 (2003).
34. C. B. Andersen *et al.*, *Nature* **443**, 663 (2006).
35. F. J. Triana-Alonso, K. Chakraborty, K. H. Nierhaus, *J. Biol. Chem.* **270**, 20473 (1995).
36. G. Z. Yusupova, M. M. Yusupov, J. H. Cate, H. F. Noller, *Cell* **106**, 233 (2001).
37. L. A. Passmore *et al.*, *Mol. Cell* **26**, 41 (2007).
38. C. M. Spahn *et al.*, *Science* **291**, 1959 (2001).
39. C. M. Spahn *et al.*, *Cell* **118**, 465 (2004).
40. A. V. Pisarev, V. G. Kolupaeva, M. M. Yusupov, C. U. Hellen, T. V. Pestova, *EMBO J.* **27**, 1609 (2008).
41. A. Korostelev *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **104**, 16840 (2007).
42. T. Kaminishi *et al.*, *Structure* **15**, 289 (2007).
43. W. L. DeLano, <http://pymol.sourceforge.net/> (2006).

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SPINE2. Coordinates and structure factors have been deposited with the Protein Data Bank with accession codes 30Z2, 3030, 3058, and 305H.

Supporting Online Material

www.sciencemag.org/cgi/content/full/330/6008/1203/DC1
Materials and Methods

Figs. S1 to S3

Tables S1 and S2

References

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REPORTS

A Magnetized Jet from a Massive Protostar

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Synchrotron emission is commonly found in relativistic jets from active galactic nuclei (AGNs) and microquasars, but so far its presence in jets from young stellar objects (YSOs) has not been proved. Here, we present evidence of polarized synchrotron emission arising from the jet of a YSO. The apparent magnetic field, with strength of ~ 0.2 milligauss, is parallel to the jet axis, and the polarization degree increases toward the jet edges, as expected for a confining helical magnetic field configuration. These characteristics are similar to those found in AGN jets, hinting at a common origin of all astrophysical jets.

Supersonic jets are observed to emerge from a wide variety of astrophysical systems, from young brown dwarfs to active galactic nuclei (AGNs). Despite their different physical scales (from hundreds to billions of astronomical units), they have strong morphological similarities (1), and a common feature of these systems is the presence of a gaseous disk around the central object. However, it is yet unclear whether there is a universal mechanism that can explain the origin of all these jets.

It is believed that the formation of stars takes place with the assistance of an accretion disk that transports gas and dust from the envelope of the system to the protostar and a jet that removes angular momentum and magnetic flux, allowing the accretion to proceed (2). Theoretical models for the formation of jets from young stellar objects (YSOs) assume that the initial acceleration takes place relatively close to the protostar as gas from the accretion disk is lifted and centrifugally

accelerated along magnetic field lines. This mechanism was first suggested as the origin of jets from accretion disks around black holes (3, 4) and was soon proposed as the mechanism for protostellar jets (5, 6). Present observational techniques cannot resolve and directly probe into the innermost regions where the jet is launched, but theoretical models for protostellar jets are able to explain some bulk observational properties, such as the correlation between accretion and ejection rates (7). The collimation of the outflow into a jet takes place at larger distances from the star and is also believed from theoretical arguments to take place magnetically. The magnetic field lines anchored in the rotating accretion disk are proposed to wrap in a helical configuration that is needed to provide the magnetic pressure to collimate the jet via “hoop” stresses (8, 9). Observations aimed at measuring the magnetic field in protostellar jets are very scarce and imply complex modeling and/or a large number of assumptions (10, 11).

A very powerful method to simultaneously obtain the structure and strength of the magnetic field in jets is through observations of the synchrotron emission. When electrons move at relativistic velocities in the presence of a magnetic field, they emit linearly polarized synchrotron radiation. The spectrum of the synchrotron emission in the centimeter wavelength range is characterized by a negative spectral index (i.e., the flux density decreases with frequency), and it is related to the strength of the magnetic field.

Additionally, the direction of the polarization is related to the direction of the magnetic field. This method has been the most successful in the study of the magnetic field in jets from AGNs (12) and microquasars (13), where the ejected material moves at relativistic velocities. In contrast, protostellar jets, where the bulk of the material moves at much smaller velocities (200 to 1000 km s⁻¹) (14, 15), apparently would lack relativistic electrons that can provide information on the magnetic field. Then, the emission is usually dominated by a different mechanism (free-free emission originated from the thermal motions of the electrons, characterized by a positive spectral index and no polarization). Intriguingly, in a few protostellar jets (associated with both low- and high-mass YSOs), radio emission with negative spectral indices, which could correspond to nonthermal synchrotron emission from relativistic electrons, has been reported [Serpens (16), HH 80-81 (17), Cepheus A (18), W3(H₂O) (19), L778 VLA 5 (20), IRAS 16547-4247 (21)] (22). If relativistic electrons were present, the acceleration of particles would be most likely produced where the fast thermal jet impacts on the surrounding medium and a strong shock wave is formed. Thus, particles might be accelerated up to relativistic energies by the Fermi mechanism (23). In order to confirm the presence of synchrotron radiation in these protostellar jets, a clear detection of linearly polarized radio emission is required. Because polarized emission is only a fraction of the continuum emission and protostellar jets are usually very weak, highly sensitive observations are required.

We obtained highly sensitive (total observation time ~ 12 hours) radio continuum images of the HH 80-81 jet using the Very Large Array (VLA) in its C configuration (24). This highly collimated radio jet is driven by the protostellar source IRAS 18162-2048, whose total luminosity is $\sim 17,000 L_{\odot}$ (where $L_{\odot}$ is the luminosity of the Sun) [if this luminosity originated in a single object, it would correspond to a zero-age main-sequence star of $\sim 10 M_{\odot}$ (where $M_{\odot}$ is the mass of the Sun) according to the stellar evolutionary models of (25)]. The radio jet consists of a chain of radio sources aligned in a southwest-northeast direction. To the southwest, it terminates in the

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optical and radio Herbig-Haro objects HH 80 and HH 81, whereas to the northeast it terminates in the radio Herbig-Haro object HH 80 N (17) (Fig. 1). With a total extension of 5.3 pc [for an assumed distance of 1.7 kpc (26)], this is one of the largest and most collimated protostellar jets known so far. Previous radio observations showed that the spectrum of the emission from the central source is characterized by a positive spectral index, suggesting that it is dominated by thermal free-free emission (17). In contrast, the negative spectral indices of the emission from HH 80-81, HH 80 N, as well as from some of the knots in the jet lobes, suggest that an additional non-thermal component could be present in these sources (17).

Our observations (Fig. 1) show that the emission of the knots located ~ 0.5 pc from the driving source is linearly polarized, indicating the presence of nonthermal synchrotron emission in this jet (24) and implying the presence of relativistic electrons and a magnetic field. The observed polarization vectors are perpendicular to the direction of the jet, with a degree of polarization of the order of 10 to 30%. The direction of the apparent magnetic field (the component in the plane of the sky averaged over the line of sight) is taken to be perpendicular to the polarization vectors [this is correct for a nonrelativistically moving source, such as the HH 80-81 jet; for relativistic jets, such as AGN jets, additional assumptions on the velocity field are required (27)]. Then, the apparent magnetic field appears very well aligned with the direction of the HH 80-81 radio jet (Fig. 1C).

By using the equations 7.14 and 7.15 of (28) we obtained the minimum total energy, $E = c_{13}(1 + k)^{4/7}\phi^{3/7}R^{9/7}L_R^{4/7}$, and the equipartition magnetic field, $B = [4.5c_{12}(1 + k)/\phi]^{2/7}R^{-6/7}L_R^{2/7}$. In these equations, ϕ is the filling factor of the emitting region, R is the radius of the source, L_R is the integrated radio luminosity, k is the ratio between the heavy particle energy and the electron energy, and c_{12} and c_{13} are functions of the spectral index and of the minimum and maximum frequencies considered in the integration of the spectrum, which are given in appendix 2 of (28). To estimate the radio luminosity, we integrated the radio spectrum in the range between 20 and 2 cm by using the flux density measurements of (17) (table S1). We used a filling factor of $\phi = 0.5$ and $k = 40$ [this value of k is appropriate for acceleration in a nonrelativistic shock (29)]. With these parameters, we obtained typical values for the knots of the radio jets of $B \approx 0.2$ mG and $E \approx 2 \times 10^{43}$ erg.

The direction of the apparent magnetic field obtained from the synchrotron emission is strongly concentrated in a direction very close ($\sim 4^\circ$) to the jet axis, whereas the direction of the magnetic field inferred from polarization of the dust near the protostar (30) is offset by $\sim 20^\circ$ and shows more scatter (Fig. 2); however, part of this scatter could be attributed to turbulent and thermal motions. It has been estimated that the magnetic field in the region ~ 0.1 pc around the protostar

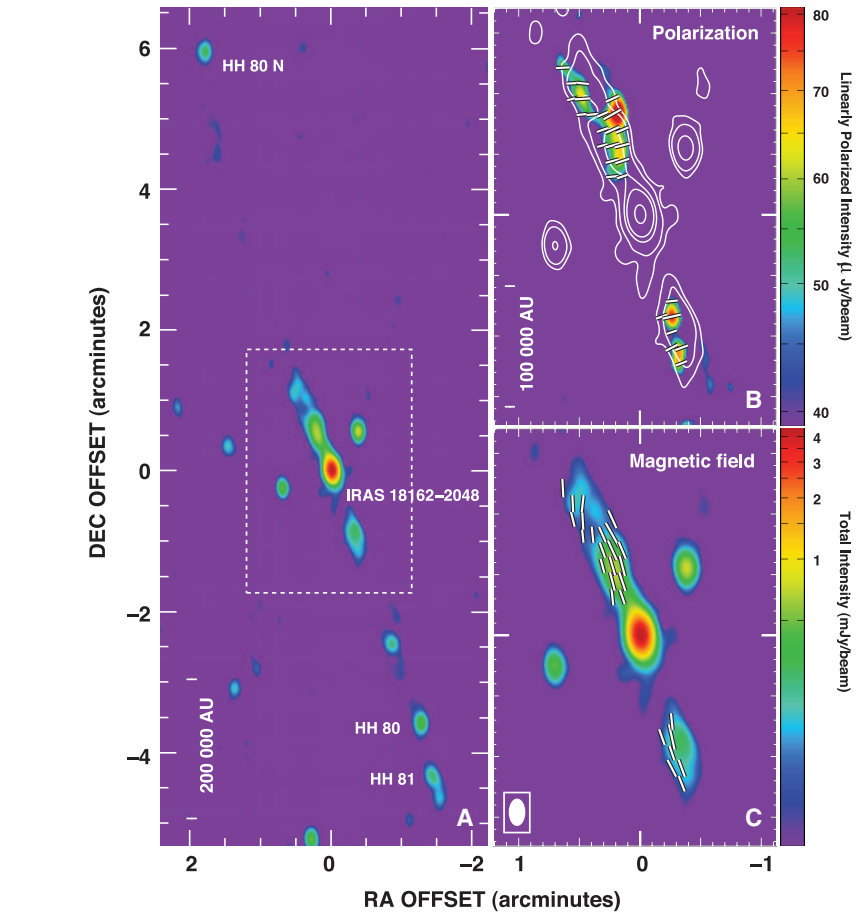


Fig. 1. Images of the HH 80-81 jet region at 6 cm wavelength. (A) Image of the total continuum intensity, showing the whole extension of the HH 80-81 jet. The brightest radio knot (labeled as IRAS 18162–2048) is associated with the central protostar. The color scale [shown in (C)] ranges from 0.039 to 4.5 mJy beam $^{-1}$. The rectangle marks the central region of the jet, which is shown in (B) and (C). (B) Linearly polarized continuum intensity image (color scale). The color scale ranges from 39 to 85 μ Jy beam $^{-1}$. Polarization direction is shown as white bars. The total continuum intensity is also shown (contours). Contour levels are 40, 100, 400, 850, and 3300 μ Jy beam $^{-1}$. (C) The apparent magnetic field direction (white bars) is superposed with the total continuum intensity image (color scale). The images were made by using a tapering of 20 k λ in order to emphasize extended emission. The root mean square of the noise of the images is 0.013 mJy beam $^{-1}$, and the synthesized beam is 13'' by 8'' with a position angle of 2° [shown in the bottom left corner of (C)]. The (0,0) offset position corresponds to the phase center of the observations, at right ascension (RA, J2000) = 18^h 19^m 12.102^s and declination (DEC, J2000) = $-20^\circ 47' 30.61''$.

IRAS 18162–2048 has a value of 0.2 mG (30). If we assume that this field is anchored to the dust envelope and behaves like a dipole, it should drop with distance cubed. However, even at ~ 0.5 pc from the protostar, the strength of the magnetic field in the jet remains comparable to that observed in the core by (30), when it should have decreased by more than a factor of 100 if it was merely reflecting the field in the ambient cloud. These results suggest that, whereas dust polarization traces the magnetic field associated with the ambient material close to the protostar, the synchrotron emission traces the magnetic field intrinsically associated with the jet.

The polarization properties and the magnetic field configuration in the HH 80-81 jet are very similar to those observed in AGN jets. In AGN jets, the polarization is always observed either

perpendicular or parallel to the axis of the jet (27). When jets are transversally resolved, the degree of linear polarization reaches a minimum toward the jet axis and increases toward the jet edges [e.g., figure 4a in (31)]. Figure 1B shows that the linear polarization in HH 80-81 is perpendicular to the jet axis, and Fig. 3 shows that the degree of linear polarization increases as a function of the distance from the jet axis. In AGN jets, this configuration of the polarization has been interpreted as indicative of a large-scale helical magnetic field (27, 31).

The relevance of magnetic fields in protostellar jets has been anticipated from theoretical models of star formation [see (4, 32, 33), and references therein]. However, most of the attention has been paid to the role of the magnetic field in the launching region, and its relevance in the

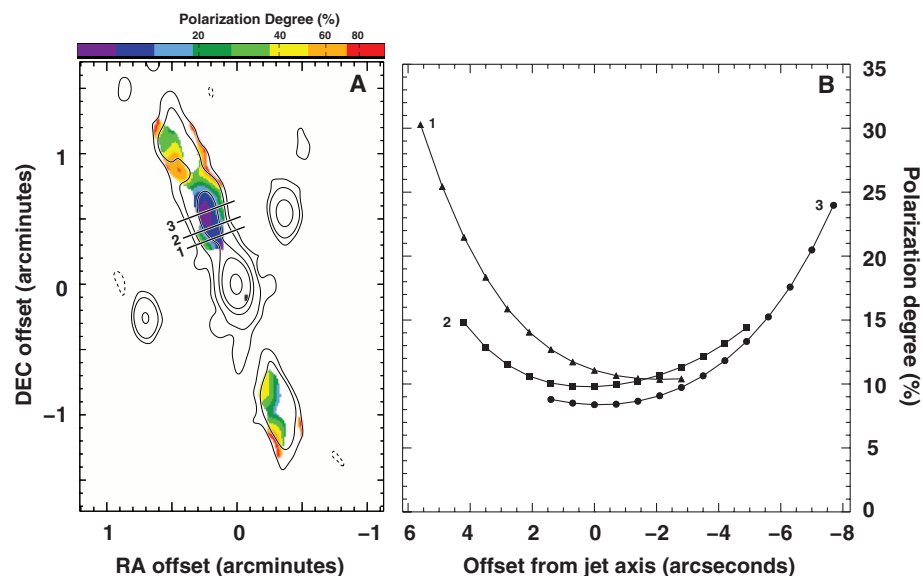
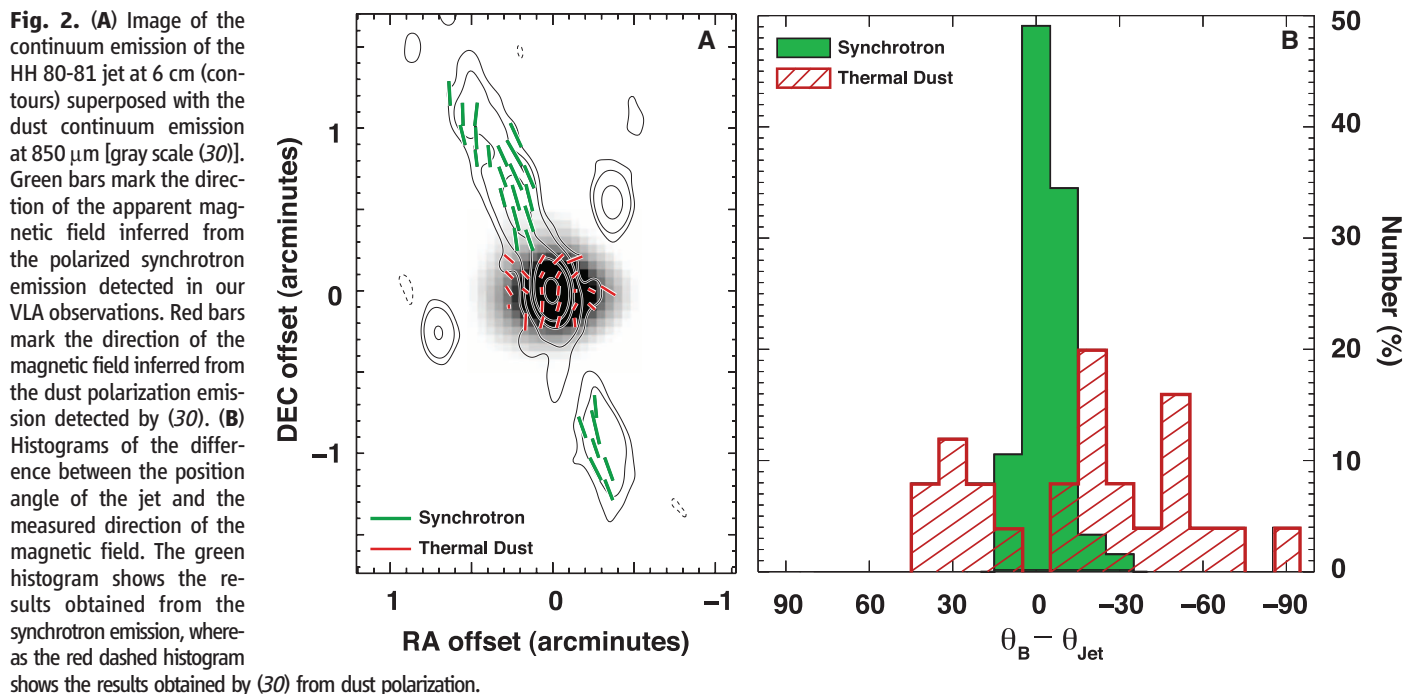


Fig. 3. (A) Image of the continuum emission of the HH 80-81 jet at 6 cm (contours), superposed with the map of polarization degree (colors), obtained as the ratio of the polarized emission over the total continuum emission. Contour levels are 40, 100, 400, 850, and 3300 $\mu\text{Jy beam}^{-1}$. The color scale ranges from 5 to 100%. Black lines mark the direction of the slices shown in (B). (B) Polarization degree for different slices across the jet.

collimation and propagation at larger scales has been scarcely studied, even theoretically (34). Possible signatures of magnetic fields on the large scales of the YSO outflows have been searched with multidimensional numerical MHD simulations of overdense, radiative cooling jets [see (35) and references therein]. These studies find that the effects of magnetic fields on the outflow are dependent on both the field geometry and the strength, which so far have been poorly determined from observations [e.g., (35)]. Our results for the HH 80-81 jet provide a measure of the

strength and structure of the magnetic field in a YSO jet at large distances (~ 0.5 pc) from the driving source. Jets from YSOs produce thermal line emission from shocks that in turn provides direct, independent information on their physical conditions (density, temperature, and velocity) (36) not available for jets from AGNs and microquasars. Our results in the HH 80-81 jet suggest that mapping of linear polarization in a set of protostellar jets (associated with both low- and high-mass YSOs) in combination with detailed theoretical modeling may lead to a

deeper understanding of the overall jet phenomenon and of the relevance of magnetic fields in star formation. Although these radio jets are usually weak, these studies will be feasible with the next generation of ultrasensitive radio interferometers. Recent molecular maser observations are starting to provide information on the strength and direction of the magnetic field in the disks around massive protostars (37); observations of the dust polarization provide information on the strength and direction of the magnetic field in the envelope feeding the disk in the protostellar system (30, 38). Thus, we may be on the brink of describing the magnetic field characteristics in the complete envelope-disk-outflow system with a combination of observational techniques.

Furthermore, recent theoretical works [e.g., (39)] suggest that protostellar jets could be a source of gamma rays. These models postulate, as a working hypothesis, the presence of relativistic electrons in such jets. Our detection of synchrotron emission in the HH 80-81 jet demonstrates the presence of relativistic electrons in the jets from massive protostars, providing an observational ground to the theoretical conjecture and making these objects a potential target for future high-energy studies.

References and Notes

1. D. S. De Young, *Science* **252**, 389 (1991).
2. This scenario is well established in the case of low-mass stars, whereas for high-mass stars the evidence of disk/jet systems is more scarce [e.g., (40)].
3. R. D. Blandford, D. G. Payne, *Mon. Not. R. Astron. Soc.* **199**, 883 (1982).
4. J. C. McKinney, R. D. Blandford, *Mon. Not. R. Astron. Soc.* **394**, L126 (2009).
5. R. E. Pudritz, C. A. Norman, *Astrophys. J.* **274**, 677 (1983).
6. F. Shu *et al.*, *Astrophys. J.* **429**, 781 (1994).
7. J. Ferreira, *Astron. Astrophys.* **319**, 340 (1997).
8. M. Livio, in *Accretion Phenomena and Related Outflows*, IAU Colloquium 163, D. T. Wickramasinghe, L. Ferrario,

- G. V. Bicknell, Eds. (ASP Conference Series Vol. 121, Astronomical Society of the Pacific, San Francisco, 1997), pp. 845–866.
9. R. E. Pudritz, R. Ouyed, Ch. Fendt, A. Brandenburg, in *Protostars and Planets V*, B. Reipurth, D. Jewitt, K. Keil, Eds. (University of Arizona Press, Tucson, AZ, 2007), pp. 277–294.
 10. A. Chrysostomou, P. W. Lucas, J. H. Hough, *Nature* **450**, 71 (2007).
 11. J. A. Morse, S. Heathcote, G. Cecil, P. Hartigan, J. C. Raymond, *Astrophys. J.* **410**, 764 (1993).
 12. A. H. Bridle, R. A. Perley, *Annu. Rev. Astron. Astrophys.* **22**, 319 (1984).
 13. I. F. Mirabel, L. F. Rodríguez, *Annu. Rev. Astron. Astrophys.* **37**, 409 (1999).
 14. J. Martí, L. F. Rodríguez, B. Reipurth, *Astrophys. J.* **449**, 184 (1995).
 15. G. Pech *et al.*, *Astrophys. J.* **712**, 1403 (2010).
 16. L. F. Rodríguez *et al.*, *Astrophys. J.* **346**, L85 (1989).
 17. J. Martí, L. F. Rodríguez, B. Reipurth, *Astrophys. J.* **416**, 208 (1993).
 18. G. Garay, S. Ramirez, L. F. Rodríguez, S. Curiel, J. M. Torrelles, *Astrophys. J.* **459**, 193 (1996).
 19. D. J. Wilner, M. J. Reid, K. M. Menten, *Astrophys. J.* **513**, 775 (1999).
 20. J. M. Girart, S. Curiel, L. F. Rodríguez, J. Cantó, *Rev. Mex. Astron. Astrofis.* **38**, 169 (2002).
 21. L. F. Rodríguez, G. Garay, K. Brooks, D. Mardones, *Astrophys. J.* **626**, 953 (2005).
 22. Circularly polarized emission has been reported in the ejecta of the young star T Tauri South (41), but in this case the proposed mechanism is gyrosynchrotron, which has different polarization characteristics than synchrotron and is expected to be relevant only close to the stellar surfaces.
 23. A. R. Bell, *Mon. Not. R. Astron. Soc.* **182**, 147 (1978).
 24. See supporting material on Science Online.
 25. G. Schaller, D. Schaerer, G. Meynet, A. Maeder, *Astron. Astrophys. Suppl. Ser.* **96**, 269 (1992).
 26. L. F. Rodríguez, J. M. Moran, P. T. P. Ho, E. W. Gottlieb, *Astrophys. J.* **235**, 845 (1980).
 27. M. Lyutikov, V. I. Pariev, D. C. Gabuzda, *Mon. Not. R. Astron. Soc.* **360**, 869 (2005).
 28. A. G. Pacholczyk, *Radio Astrophysics* (Freeman, San Francisco, 1970).
 29. R. Beck, M. Krause, *Astron. Nachr.* **326**, 414 (2005).
 30. R. L. Curran, A. Chrysostomou, *Mon. Not. R. Astron. Soc.* **382**, 699 (2007).
 31. J. L. Gómez, A. P. Marscher, S. G. Jorstad, I. Agudo, M. Roca-Sogorb, *Astrophys. J.* **681**, L69 (2008).
 32. S. Gabrit, *Int. Astron. Union Symp.* **243**, 203 (2007).
 33. S. Lizano, F. H. Shu, D. Galli, A. Glassgold, *Rev. Mex. Astron. Astrof. Ser. Conf.* **36**, 149 (2009).
 34. T. P. Ray, *Rev. Mex. Astron. Astrof. Ser. Conf.* **36**, 179 (2009).
 35. E. M. de Gouveia dal Pino, *AIP Conf. Proc.* **784**, 183 (2005).
 36. B. Reipurth, J. Bally, *Annu. Rev. Astron. Astrophys.* **39**, 403 (2001).
 37. W. H. T. Vlemmings, G. Surcis, K. J. E. Torstensson, H. J. van Langevelde, *Mon. Not. R. Astron. Soc.* **404**, 134 (2010).
 38. J. M. Girart, R. Rao, D. P. Marrone, *Science* **313**, 812 (2006).
 39. V. Bosch-Ramon, G. E. Romero, A. T. Araudo, J. M. Paredes, *Astron. Astrophys.* **511**, A8 (2010).
 40. N. A. Patel *et al.*, *Nature* **437**, 109 (2005).
 41. T. P. Ray *et al.*, *Nature* **385**, 415 (1997).
 42. National Radio Astronomy Observatory is a facility of the NSF, operated under cooperative agreement by Associated Universities, Incorporated. We acknowledge I. Agudo, A. Alberdi, and J. L. Gómez for useful comments. G.A., C.C.-G., J.M., M.O., and J.M.T. acknowledge support from Ministerio de Ciencia e Innovación (Spain) grants AYA2008-06189-C03 and AYA2007-68034-C03-02 [co-funded with Fondo Europeo de Desarrollo Regional (FEDER) funds] and from Junta de Andalucía (Spain). L.F.R. acknowledges the support of Dirección General de Asuntos del Personal Académico, UNAM, and Consejo Nacional de Ciencia y Tecnología, México.

Supporting Online Material

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Spin-Light Coherence for Single-Spin Measurement and Control in Diamond

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The exceptional spin coherence of nitrogen-vacancy centers in diamond motivates their function in emerging quantum technologies. Traditionally, the spin state of individual centers is measured optically and destructively. We demonstrate dispersive, single-spin coupling to light for both nondestructive spin measurement, through the Faraday effect, and coherent spin manipulation, through the optical Stark effect. These interactions can enable the coherent exchange of quantum information between single nitrogen-vacancy spins and light, facilitating coherent measurement, control, and entanglement that is scalable over large distances.

The coherent coupling of light with matter provides a powerful tool for quantum measurement and control. The resulting hybrid states have been employed in individually addressable semiconducting (1), atomic (2), and superconducting (3) quantum systems. Individual diamond nitrogen-vacancy (NV) centers (4) show great promise as solid-state spin qubits with demonstrations of millisecond coherence times (5) and nanosecond manipulation times (6). NV-center spins coherently couple to nearby electronic (7, 8) and nuclear (9, 10) spins, creating few-qubit networks for simple algorithms and quantum memories. NV-center spin states are conventionally read out destructively with the use of spin-dependent photoluminescence (PL). We present nondestructive single-spin measurement via the Faraday effect (FE) and unitary single-spin manipulation via the

optical Stark effect (OSE) with the use of a near-resonant laser field coupled to an NV center. With enhanced coupling from an optical cavity, these techniques can be extended to quantum weak and quantum nondemolition measurements of single spins, facilitating applications such as quantum repeaters (11) and spin-photon entanglement (12) on demand. This approach may also assist with the study of newly identified defect-based spin systems (13) with limited single-spin read-out alternatives.

The FE and OSE probe the respective light and spin responses to the near-resonant coupling between an NV center and light, which emerge from spin-dependent optical transitions (14). Each optical transition's ground ($|g\rangle$) and excited ($|e\rangle$) states coherently mix with the laser field (Fig. 1A), forming polariton states ($|G\rangle$ and $|E\rangle$) whose energies repel in the form of a level anticrossing (Fig. 1A, inset) as a function of the energy detuning (Δ) between the light and the optical transition. The groundlike polariton states ($|G\rangle$) adiabatically remain occupied as they shift in energy during the interaction. The FE originates from polarization

selection rules in which the coupled polarization component of the coherent optical field experiences a spin-dependent phase shift relative to the other. Simultaneously, the OSE energy shifts add quantum phases to the spin states producing spin rotation. Theoretical predictions of the FE and OSE from a modified Jaynes-Cummings model are in quantitative agreement with our measurements (15).

A diamond NV center consists of a vacancy adjacent to a substitutional nitrogen atom in the diamond lattice. When negatively charged, the orbital ground state is a spin triplet whose levels function as a qubit (4) and are tunable with an applied magnetic field. Microwave magnetic fields that are resonant and near-resonant (16) with the spin transitions produce unitary rotations of the spin state. NV centers exhibit spin-conserving optical transitions (Fig. 1B) to an orbital-doublet, spin-triplet excited state 1.945 eV above the ground state (17). An NV center may be optically excited into the excited state, either resonantly at 637 nm or with higher-energy light along with phonon creation. Phonons can also be created during spontaneous emission back to the ground state, resulting in red-shifted PL. Optical excitation causes spin polarization from the $m_s = \pm 1$ spin states into the $m_s = 0$ spin state through an intersystem-crossing decay mechanism. PL is inhibited during the 460-ns cryogenic lifetime (18) of this decay, resulting in a spin-dependent PL intensity that is used for destructive spin-projection measurements. These dynamics allow individual NV centers to be optically addressed.

The NV-center spin coherently couples to light in our measurements because the optical transition energies (Fig. 1C) vary by spin state as a result of spin-spin and spin-orbit interactions (19). These transitions are energetically stable

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(20) in pure diamond hosts under cryogenic temperatures (21). In our NV centers, the transverse crystal strain is strong enough to energetically separate the excited-state orbital doublet into two branches with approximately linear, orthogonal optical-transition dipoles aligned to the transverse strain: 3E_X and 3E_Y (22). These dipoles couple to orthogonal near-linear polarizations of light, giving rise to the FE in a nonstandard polarization basis. A magnetic field is applied along the NV symmetry axis to produce similar spin eigenstates in both the ground and excited orbitals, providing robust spin-conserving optical transitions. In the range of crystal strains and magnetic fields that we studied, the largest optical transition-energy spin splitting occurs in the lower-energy excited-state orbital branch (3E_Y). The 3E_Y , $m_s = -1$ optical transition is roughly 3 GHz lower in energy than the 3E_Y , $m_s = 0$ optical transition (Fig. 1C, right). The fine structure of the higher-energy excited state orbital branch (3E_X) more closely resembles the ground-state fine structure for our experimental conditions, producing spin-conserving optical transitions that are closely spaced in energy. The spin splitting in the 3E_Y branch is insensitive to changes in strain and magnetic field when these effects dominate spin-orbit interactions, allowing the spin-light interactions investigated here to persist across a broad range of experimental conditions.

We assembled a confocal microscope to study single NV centers at cryogenic temperatures. The NV centers were naturally formed in high-purity diamond grown by chemical vapor deposition specified to contain less than 5 parts per billion nitrogen impurities. A 0.85-numerical aperture microscope objective focused both a red laser, tunable in energy across the NV-center optical transitions at 637 nm, and a green 532-nm laser onto a single NV center for both resonant and nonresonant optical excitation, respectively. PL from the NV center's phonon sideband was collected with the objective and detected with an avalanche photodiode (APD). The PL provided two important measurements: (i) the spin projection ($\langle S_Z \rangle$) under green illumination and (ii) resonant optical absorption from photoluminescence excitation (I_{PLE}) under tunable red excitation. We used a lithographically patterned, short-terminated coplanar waveguide (6) to apply microwave-frequency magnetic fields for resonant spin manipulation of the ground-state triplet. The lasers, microwave fields, and measurements of PL were all gated for time-domain measurements. The polarization of the tunable red laser light was prepared relative to the orthogonal optical dipoles of the NV center, ensuring that each dipole interacted with half of the light for polarization-interference measurements. The transmitted red laser light was collected through a back-side window in the cryostat, passed through polarization optics to project the correct polarization, and then analyzed with a near shot-noise-limited balanced photodiode bridge whose signal was measured with a lock-in amplifier (15).

We studied the FE spin dependence and the corresponding NV-center response to a 1- μ s pulse of tunable red laser light as a function of the light's energy. In the measurement sequence (Fig. 2A), the initial spin state was prepared alternately in either $m_s = 0$ or $m_s = -1$ before the red laser light pulse. The measured spin-dependent FE (Φ_F) is the difference in phase shift of the red laser light polarization between the two spin preparations. Additionally, both I_{PLE} and $\langle S_Z \rangle$ were measured for both prepared spin states with PL measured at different times during the sequence. To increase the signal-to-noise ratio, Φ_F , I_{PLE} , and $\langle S_Z \rangle$ data were averaged for $\sim 5 \times 10^6$ repeated measurements, requiring ~ 30 s per point. These three independent measurements were correlated as the red laser light energy was tuned across the optical transitions, providing a detailed picture of the full interaction dynamics.

To illustrate the FE dynamics in our system, simultaneous measurements of Φ_F , I_{PLE} , and $\langle S_Z \rangle$ at a temperature of 8 K and an applied magnetic field of 1620 G are shown for the lower-energy E_Y orbital-branch optical transitions at red laser powers of 5 and 0.1 μ W (Fig. 2, B and C, respectively). The E_Y , $m_s = 0$ optical transition (circled in Fig. 1C) defines the origin of the red laser energy scale, placing the E_Y , $m_s = -1$ optical transition near -3-GHz laser energy in all presented FE data. As a result of spectral broadening of

the optical transitions (21), Φ_F is also broadened, resulting in a reduced response near resonances. The $m_s = \pm 1$ spin states are susceptible to polarization into the $m_s = 0$ spin state when optically excited because of the intersystem-crossing decay, which is measured directly with $\langle S_Z \rangle$. Therefore, optical spin polarization reduces Φ_F and I_{PLE} signals more substantially near resonance with $m_s = \pm 1$ optical transitions and at higher powers. Photo-ionization (23) may also result from red laser excitation, which reduces Φ_F and I_{PLE} signals and skews $\langle S_Z \rangle$. The NV center is less perturbed at lower red laser powers, producing more symmetric FE data at smaller signal intensities (Fig. 2C). The temperature dependence of the FE that we measured (15) was consistent with previous studies of the excited-state energetic stability with temperature (21). Using our developed model (15) along with our measured FE response, we determined that the average, equal-intensity area of the red laser confocal spot was $(0.75 \pm 0.1 \mu\text{m})^2$, which is consistent with diffraction, drift, and aberration limits.

FE measurements showing both E_X and E_Y orbital-branch optical transitions at a temperature of 8 K, an applied magnetic field of 1920 G, and a higher red laser power of 15 μ W are shown in Fig. 2D. The $m_s = 0$ and $m_s = -1$ optical transitions in the E_X orbital branch (near 16.5-GHz laser energy) are closer together in energy than

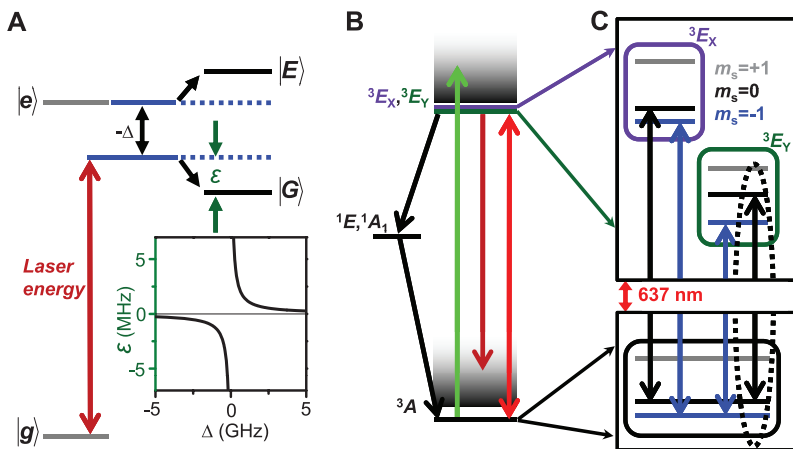


Fig. 1. (A) An optical transition interacting with a laser detuned in energy by Δ relative to the transition energy. Under adiabatic conditions, the spin-light polariton energy levels shift by ϵ as a result of the interaction, producing measurable effects in both the phase of interacting light and the NV-center spin dynamics. (Inset) ϵ as a function of Δ . (B) NV-center energy-level diagram depicting ground (3A) and excited (3E_X , 3E_Y) spin triplet levels. From left to right: Diagonal arrows show the intersystem crossing from the excited $m_s = \pm 1$ states to the ground $m_s = 0$ state through singlet-level (1E , 1A_1) metastable decay, which permits spin initialization and measurement. The nonresonant green upward and red downward arrows depict optical excitation and collected PL, respectively. The red double arrow depicts the multiple resonant transitions magnified in (C). (C) Fine structure of the presented NV-center ground- and excited-state energy levels at 1920 G, additionally depicting spin-conserving optical transitions for $m_s = 0$ (black arrows) and $m_s = -1$ (blue arrows) spin states in both excited-state orbitals. The E_Y , $m_s = 0$ (circled) spin-conserving transition is ~ 3 GHz larger in energy than the E_Y , $m_s = -1$ transition for our measurements. With increased strain, 3E_X and 3E_Y orbital energies further separate. An increase in magnetic field shifts the $m_s = +1$ and $m_s = -1$ spin sublevels up and down, respectively, in all orbital branches, having only an indirect effect on changing spin-conserving optical transition energies.

their spectrally broadened absorption and spin polarization widths, making them difficult to differentiate and reducing Φ_F for this orbital branch. Optical coupling to the E_X orbital branch is 40% weaker relative to the E_Y orbital branch due to the NV center's orientation, further reducing Φ_F for the E_X orbital branch. The E_X orbital branch picks up a stronger $m_s = 0$ optical transition response in Φ_F , probably because the $m_s = -1$ spin decays more easily through the intersystem crossing, which inhibits the FE during its lifetime. Data from the E_Y orbital-branch optical transitions in Fig. 2D resemble those of Fig. 2, B and C, but at a higher power, showing an increased asymmetry between the $m_s = 0$ and $m_s = -1$ optical transitions. Optical transitions that do not conserve the spin state can also be observed at this red laser power. Black arrows between I_{PLE} and $\langle S_Z \rangle$ graphs denote the four spin-conserving transition energies, with the E_X transitions being nearly degenerate. Blue and red arrows denote the expected positions of spin-nonconserving transitions coupled to the $m_s = 0$ and $m_s = -1$ ground spin states, respectively. Sharp features in both I_{PLE} and $\langle S_Z \rangle$ are consistent with these spin-nonconserving optical transition energies but do not line up exactly with their expected positions because the hysteretic and nonlinear response of the red laser energy tuning was not actively corrected. FE measurements on a different NV center gave nearly identical results for both E_X and E_Y orbital branches, although these orbital transitions were further separated in energy as a result of higher crystal strain.

Complementary to the light's FE response, the NV-center spin also coherently reacts to its dispersive interactions with light in the form of the OSE. The OSE manifests as relative energy shifts of the ground spin eigenstates, which alter the spin's Larmor precession rate during interaction with the red laser light pulse. This process is equivalent to a σ_Z spin rotation of the spin qubit in the Bloch sphere rotating frame representation. We measured the OSE with the use of a modified Hahn echo microwave pulse sequence (Fig. 3A) to mitigate slow environmental dephasing. The red laser interaction was timed between the two final Hahn microwave pulses such that the OSE spin evolution is encoded in the final spin state that was measured with PL intensity (I_{PL}). Through phase control of the final Hahn echo pulse, we measured multiple spin projections to give a more detailed picture of the OSE spin dynamics. Figure 3B shows the OSE spin rotation as a function of red laser pulse duration. All OSE spin projection data were averaged over 2.5×10^5 repeated measurements, taking 2.6 s for each data point and ~ 15 min for typical scans. An exponentially decaying sinusoidal fit to these data determines the Larmor frequency shift (Σ_S) induced by the OSE for fixed red laser energy and power. When the laser detuning is much larger than the resonant optical Rabi frequency, Σ_S is nearly linear with laser power (Fig. 3C). We determined the ground-state spin coherence to be

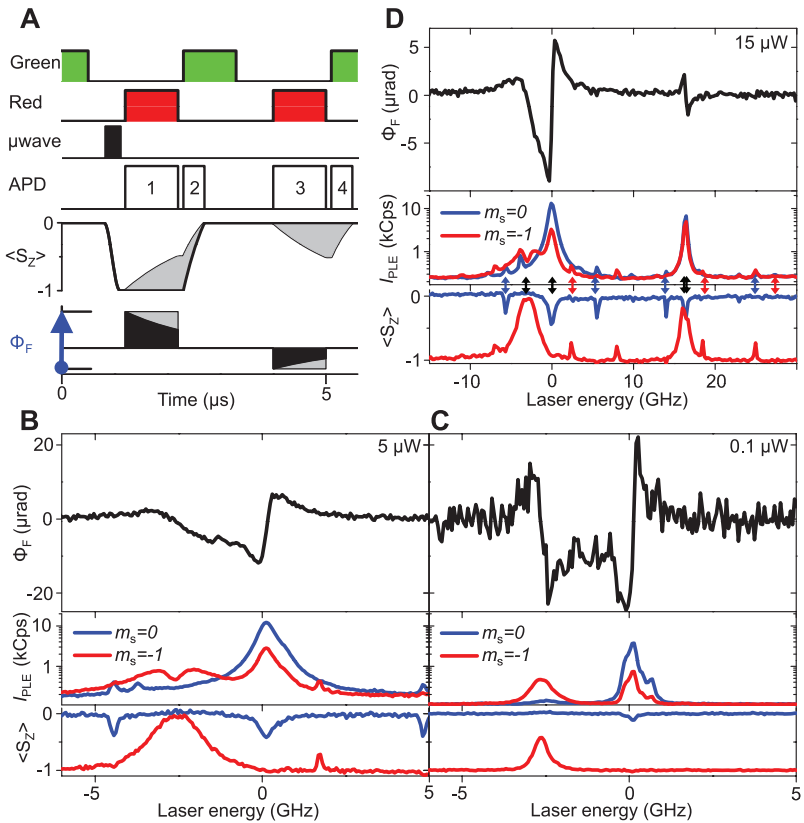


Fig. 2. (A) FE measurement timing sequence showing the gated green laser, tunable red laser, microwave, and multiplexed APD timing, as well as the spin-state evolution and diode bridge signal in time. PL photons are binned separately in time to measure both I_{PLE} (APD bins 1, 3) and $\langle S_Z \rangle$ (APD bins 2, 4) for the $m_s = -1$ and $m_s = 0$ prepared spin states. Gray areas indicate possible spin-polarization effects from the red laser. Φ_F (denoted by the blue arrow at bottom-left) is the difference in measured red laser polarization response between the prepared $m_s = 0$ and $m_s = -1$ spin states. **(B and C)** FE data sets scaled identically showing Φ_F , as well as I_{PLE} and $\langle S_Z \rangle$, for both $m_s = 0$ (blue) and $m_s = -1$ (red) prepared spin states of the 3E_Y orbital-branch optical transitions at 5 and 0.1 μW red laser power, respectively. The $m_s = 0$ optical transition (at 0-GHz laser energy) is more robust against spin polarization than the $m_s = -1$ optical transition (near -3 -GHz laser energy). **(D)** FE data set at 15 μW scanning across both 3E_X and 3E_Y orbital-branch optical transition energies. The measured FE is substantially reduced for the 3E_X orbital transitions (near 16.5 GHz), primarily as a result of the smaller optical transition energy splitting between the spin states. Vertical-pointing double arrows denote expected transition energies, as described in the text.

$T_2 = 480 \mu\text{s}$ with Gaussian decay using a Hahn echo sequence without applying the OSE, revealing that pure spin decoherence is a negligible effect in the OSE data.

By comparing the FE and OSE responses together as a function of red laser energy, we analyzed both light and spin components of the polariton dynamics, which gives deeper insight into the full NV-center spin interactions with coherent light. Φ_F and Σ_S data are superimposed as a function of laser energy at a temperature of 8 K, an applied magnetic field of 1920 G, and a red laser power of 0.66 μW (Fig. 3D, top). By fitting Σ_S as a function of laser energy, the resonant optical Rabi frequency was indirectly determined to be $2\pi \times 70$ MHz at this laser power. The relation between Φ_F and Σ_S gives information regarding the mean photon number of the interaction (I), which can be used to calibrate the

response to stronger light coupling, down to the single-photon level.

The decay of the OSE oscillations present in Fig. 3B can be analyzed in terms of dephasing between the repeated interrogations of the OSE-shifted spin. The number of OSE oscillations to $1/e$ exponential decay (N_D) in Fig. 3E is plotted as a function of laser energy for two laser powers. The smallest number of coherent oscillations occurs near the optical resonances where absorption, spin polarization (Fig. 3D, bottom), and spectral diffusion become most prominent. Although there are probably several sources of fundamental decoherence and experimental dephasing, these data qualitatively agree with the red line in Fig. 3E, which is a fit using a model that includes only two contributions (I): first, dephasing from spectral hopping giving a distribution of transition energies, and second, dephasing from the red laser

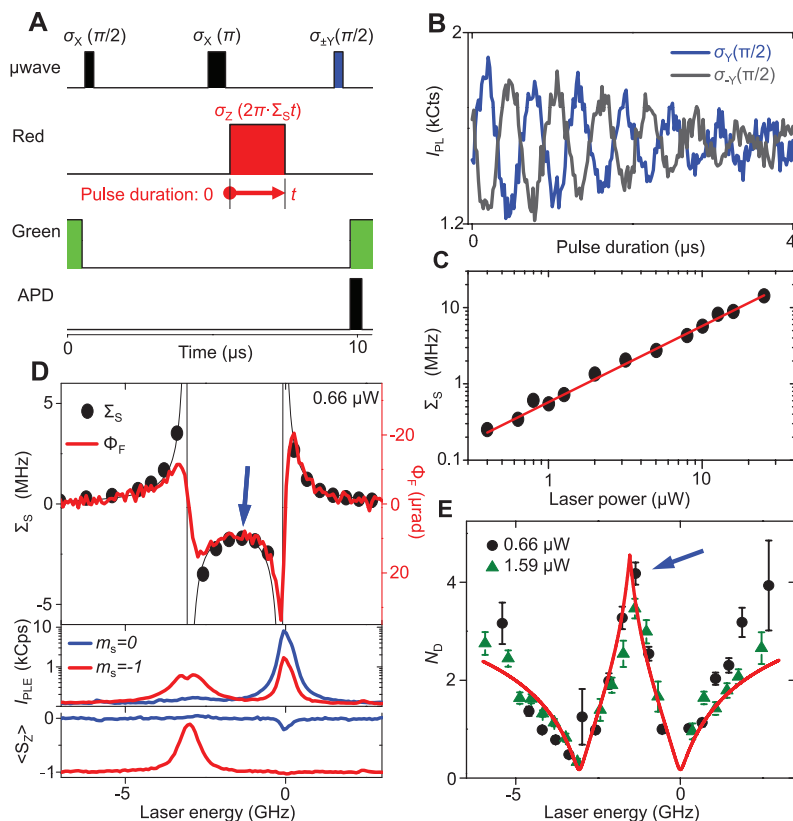


Fig. 3. (A) OSE measurement timing sequence. The red laser produces a σ_z spin rotation proportional to its 0- to 4- μ s pulse duration. $\langle S_z \rangle$ follows $\pm \sin(\sigma_z)$ as a result of a $\pm 90^\circ$ microwave phase shift of the final Hahn echo pulse. (B) I_{PL} of sequences that have these two microwave phases. kCts, photon kilocounts. (C) Σ_s as a function of laser power at ~ 2 -GHz laser energy. The error is represented by the data-point size and is dominated by the 15% uncertainty in the laser power calibration. The red line is a linear fit of Σ_s with 5.74 MHz per microwatt slope. (D) Comparison of Σ_s and Φ_F showing their complimentary response as a function of laser energy and also showing I_{PLE} and $\langle S_z \rangle$ taken with Φ_F for both prepared spin states. OSE and FE data sets were taken under the same experimental conditions at 0.66- μ W laser power. The Σ_s data point denoted by the blue arrow is the frequency fit of the data presented in Fig. 3B. (E) OSE spin coherence measured in number (N_D) of σ_z rotations to the $1/e$ decay point. Black circles represent the measurements with 0.66- μ W red laser power (Fig. 3D), and green triangles represent measurements with 1.59- μ W red laser power. The blue arrow denotes the N_D data point fit from in Fig. 3B. The red line is a fit to the data using a model incorporating dephasing from spectral broadening and laser intensity fluctuations. Error bars indicate standard errors of OSE data fits.

intensity fluctuations. The fit yields a spectral hopping half width at half maximum of 63 ± 13 MHz and laser power fluctuations of $3.5 \pm 0.8\%$. These values, along with the 71-MHz power-broadened natural linewidth, are consistent with the 150-MHz I_{PLE} -measured linewidth of the $m_s = 0$ optical transition shown in Fig. 3D. This suggests that spectral diffusion and local laser intensity fluctuations play primary roles in the decay of the OSE oscillations, consistent with recent studies of optical Rabi oscillations (24). The cumulative fidelity for a π spin rotation in Fig. 3B is $89 \pm 1\%$ with resistance to back-action spin flips of $98.9 \pm 0.3\%$ (15). These two fidelities set limits on the expected fidelity of either single-spin/single-photon entanglement generation or quantum nondemolition measurements, given an appropriately designed cavity to enhance the per-photon spin-light interaction under otherwise sim-

ilar conditions. Improvements in the experimental design and material quality may mitigate the external sources of dephasing and lead to even higher-fidelity spin-light coupling for future applications of NV centers in diamond.

The coherent coupling between an NV-center spin and light is a critical advancement for the use of NV centers in quantum information science. For example, by mixing the spin eigenstates in either the ground or excited orbitals to form a V (25) or Λ (26, 27) configuration, respectively, all-optical spin manipulation and measurement could be performed in other spin bases to span the full Bloch sphere. With the increased coupling offered by photonic cavities (28), these techniques enable optical quantum nondemolition measurements (29) of single electron spins for fundamental studies of light-matter interactions, potentially leading to solid-state implementations of quan-

tum key distribution (30). Furthermore, these hybrid light-spin states could controllably couple distant NV-center spins through photonic networks (31) for increasingly complex quantum information processing operations.

References and Notes

1. J. Berezovsky, M. H. Mikkelsen, N. G. Stoltz, L. A. Coldren, D. D. Awschalom, *Science* **320**, 349 (2008).
2. S. A. Aljunied *et al.*, *Phys. Rev. Lett.* **103**, 153601 (2009).
3. A. Lupașcu *et al.*, *Nat. Phys.* **3**, 119 (2007).
4. F. Jelezko, T. Gaebel, I. Popa, A. Gruber, J. Wrachtrup, *Phys. Rev. Lett.* **92**, 076401 (2004).
5. G. Balasubramanian *et al.*, *Nat. Mater.* **8**, 383 (2009).
6. G. D. Fuchs, V. V. Dobrovitski, D. M. Toyli, F. J. Heremans, D. D. Awschalom, *Science* **326**, 1520 (2009); 10.1126/science.1181193.
7. R. Hanson, V. V. Dobrovitski, A. E. Feiguin, O. Gywat, D. D. Awschalom, *Science* **320**, 352 (2008); 10.1126.1155400.
8. P. Neumann *et al.*, *Nat. Phys.* **6**, 249 (2010).
9. L. Childress *et al.*, *Science* **314**, 281 (2006); 10.1126/science.1131871.
10. P. Neumann *et al.*, *Science* **329**, 542 (2010); 10.1126/science.1189075.
11. L. Childress, J. M. Taylor, A. S. Sørensen, M. D. Lukin, *Phys. Rev. Lett.* **96**, 070504 (2006).
12. E. Togan *et al.*, *Nature* **466**, 730 (2010).
13. J. R. Weber *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **107**, 8513 (2010).
14. C. Cohen-Tannoudji, S. Reynaud, *J. Phys. B* **10**, 345 (1977).
15. Materials and methods and theoretical analysis are available as supporting material on Science Online.
16. C. Wei, N. B. Manson, J. P. D. Martin, *Phys. Rev. Lett.* **74**, 1083 (1995).
17. N. B. Manson, J. P. Harrison, M. J. Sellars, *Phys. Rev. B* **74**, 104303 (2006).
18. V. M. Acosta, A. Jarmola, E. Bauch, D. Budker, preprint available at <http://arxiv.org/abs/1009.0032v2>.
19. A. Batalov *et al.*, *Phys. Rev. Lett.* **102**, 195506 (2009).
20. P. Tamarat *et al.*, *Phys. Rev. Lett.* **97**, 083002 (2006).
21. K.-M. C. Fu *et al.*, *Phys. Rev. Lett.* **103**, 256404 (2009).
22. F. Kaiser *et al.*, preprint available at <http://arxiv.org/abs/0906.3426v1>.
23. N. B. Manson, J. P. Harrison, *Diamond Relat. Mater.* **14**, 1705 (2005).
24. L. Robledo, H. Bernien, I. v. Weperen, R. Hanson, *Phys. Rev. Lett.* **105**, 117403 (2010).
25. H. Kosaka *et al.*, *Nature* **457**, 702 (2009).
26. C. Santori *et al.*, *Opt. Express* **14**, 7986 (2006).
27. P. Tamarat *et al.*, *N. J. Phys.* **10**, 045004 (2008).
28. P. E. Barclay, K.-M. Fu, C. Santori, R. G. Beausoleil, *Opt. Express* **17**, 9588 (2009).
29. P. Grangier, J. A. Levenson, J.-P. Poizat, *Nature* **396**, 537 (1998).
30. J.-J. Chen, J.-H. An, M. Feng, G. Liu, *J. Phys. At. Mol. Opt. Phys.* **43**, 095505 (2010).
31. A. Imamoglu *et al.*, *Phys. Rev. Lett.* **83**, 4204 (1999).
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The Evolution of Maximum Body Size of Terrestrial Mammals

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The extinction of dinosaurs at the Cretaceous/Paleogene (K/Pg) boundary was the seminal event that opened the door for the subsequent diversification of terrestrial mammals. Our compilation of maximum body size at the ordinal level by sub-epoch shows a near-exponential increase after the K/Pg. On each continent, the maximum size of mammals leveled off after 40 million years ago and thereafter remained approximately constant. There was remarkable congruence in the rate, trajectory, and upper limit across continents, orders, and trophic guilds, despite differences in geological and climatic history, turnover of lineages, and ecological variation. Our analysis suggests that although the primary driver for the evolution of giant mammals was diversification to fill ecological niches, environmental temperature and land area may have ultimately constrained the maximum size achieved.

For the first 140 million years of their evolutionary history, mammals were small and occupied a fairly narrow range of body sizes and niches (1, 2). Although diverse feeding adaptations evolved by the middle Mesozoic, and larger mammals may have preyed on small dinosaurs (3, 4), their body size range extended only from ~3 to 5 g to ~10 to 15 kg (4, 5). This restricted range almost certainly constrained the ecological roles of early mammals in paleocommunities. For example, herbivory was probably limited; allometric, anatomical, and physiological constraints set a lower threshold of ~5 kg for ruminant herbivores (6). The Cretaceous/Paleogene (K/Pg) mass extinction, which eliminated non-avian dinosaurs as well as many vertebrate, plant, and invertebrate taxa, was followed by a wholesale reorganization of ecological communities

(7). It marked the onset of rapid morphological, ecological, and phylogenetic diversification in terrestrial mammals that led to an expansion in mass by four orders of magnitude and the occupation of a full range of ecological roles (8).

Here we analyze maximum size of terrestrial mammals across different continents, taxonomic groups, phylogenetic lineages, and feeding guilds. We compiled and analyzed data on the maximum body size of each taxonomic order in each sub-epoch on each continent over their entire evolutionary history (9). Information about body mass was obtained for fossil taxa from primary sources or estimated directly from taxon-specific allometric regressions based on measurements of teeth or limbs (table S1). Because of taphonomic considerations, we focused on the maximum size achieved by each order; it tends to be reported in the literature and is robustly related to the overall body size distribution and hence to the mean and median body size (10). Fossil ages were standardized using the midpoint for each Cenozoic sub-epoch on the Gradstein geological time scale (11). Diversity estimates were extracted from the Paleobiology Database (12), using the range-through option for each interval of time. We conducted simulations to assess the potential effect of sampling on the probability of detecting the largest mammal; including as few as 10% of fossil sites yielded nearly 100% probability of recovering the largest mammal on a continent (fig. S1).

The data show that the pattern of body size evolution was similar across continents, lineages, and trophic groups. Globally, and on each continent, maximum body mass increased rapidly during the early Cenozoic (Fig. 1). By the late Eocene [42.9 million years ago (Ma)], maximum body mass was three orders of magnitude larger than at the beginning of the Cenozoic. Our results are consistent with a previous analysis of North American mammals (5, 8). The upper limit of ~17 tons was reached in the early Oligocene of

Eurasia, with the evolution of *Indricotherium transouralicum* (Perissodactyla) and again in the Miocene by several *Deinotherium* species (Proboscidea) in Eurasia and Africa (Fig. 1B; fig. S2); North America never supported a mammal of this size. Strikingly, the overall pattern was not driven by a single taxon or an individual continent. At one time or another, six different orders and three of the four continents contained the largest mammal. Because of the current paucity of data for South America, body mass values for this continent should be considered an underestimate; nonetheless, results illustrate the same general trends. Contrary to earlier suggestions (13–15), increases in body mass were not driven by increasing generic or ordinal diversity: Mammals were not consistently larger when they were more diverse (9) (fig. S3).

We tested two hypotheses for the evolution of maximum body size. The first is a simple growth model, in which maximum body size (M) evolves following a geometric Brownian motion, that is, an unconstrained random walk on the logarithmic scale. This model implicitly assumes that niche space is uniformly distributed. Under a random walk, M is predicted to increase as a power law of the form $\log M = M_0 t^\gamma$, where M_0 is initial maximum body size, t is time, and $\gamma = 1/2$, so that maximum body size increases as the square root of time (15).

The second model has growth saturating over time, reflecting limits of resources or physiological, allometric, biomechanical, or ecological constraints, such as the slower life histories of larger mammals. Thus, the initial change in body mass M with time is proportional to body mass (that is, $\frac{dM}{dt} \propto M$) and increases at some intrinsic rate α . However, as maximum body size evolves, the evolutionary possibilities for increasing size are progressively exhausted. Consequently, the rate of change is also proportional to the availability of open niche space, which is captured by the difference between asymptotic (K) and current log body mass [that is, $\log(K) - \log(M)$], or $\log\left(\frac{K}{M}\right)$. Combining these ecological and evolutionary growth dynamics yields the Gompertz equation $\frac{dM}{dt} = \alpha M \log\left(\frac{K}{M}\right)$, a sigmoidal growth model often used in time series analyses. The integrated

form is $\log M = \log K - \log\left(\frac{K}{M_0}\right)e^{-\alpha t}$, where M_0 is initial maximum body size. The Gompertz model is more biologically plausible than the random walk model, because it captures both the multiplicative nature of body size evolution and the saturating effects of exponentially decreasing niche space availability at larger body sizes.

We compared model fits using corrected Akaike information criteria (AICc). The results suggested that the random walk was not an appropriate model (Table 1). Although a power function provided a reasonable fit to the data, the fitted exponent γ was 0.25, significantly less than the predicted value of 0.50. Moreover, after the initial growth phase, the residuals were not normally distributed. This was probably because maximum

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body size approaches a plateau as opposed to increasing monotonically. The Gompertz model provided a much better fit to the data throughout the time series and yielded the lowest AICc (Table

1 and fig. S2). The inflection point between the growth phase and the saturating phase occurred during the late Eocene at 42.9 Ma, at a body mass of 4850 kg.

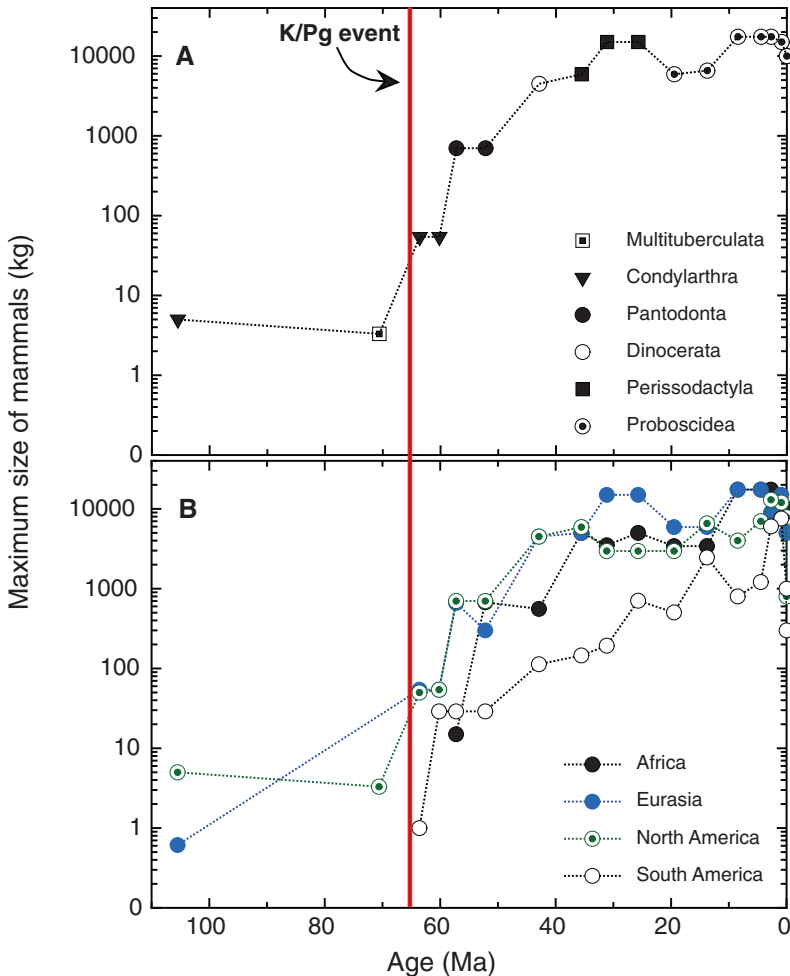


Fig. 1. Maximum body mass of terrestrial mammals over time and space. **(A)** Maximum body mass over time examined globally at the sub-epoch level over the past 110 million years. **(B)** Maximum body mass for the largest continents (South America, North America, Africa, and Eurasia) over the same time interval. The overall trend is not driven by a single taxonomic order or an individual continent; six different orders and three of the four continents depicted have at one time or another housed the largest mammal. Data for Australia (not shown) and South America were particularly difficult to obtain because of limited material and/or collecting; thus, estimates for these continents should be considered underestimates. Data are binned at the resolution of sub-epochs using the Gradstein time scale (12).

Table 1. Model fits for global, continental and trophic level body size trajectories. The power law is of the form $\log M = c_0 t^\gamma$ and the Gompertz equation $\log M = \log K - \log\left(\frac{K}{M_0}\right)e^{-\alpha t}$.

Model	Parameters	AICc	R ² value	P value
All data				
Power law	$c_0 = 1.504, \gamma = 0.25$	9.3	0.92	<0.001
Gompertz	$K = 13182.57, M_0 = 6.92, \alpha = 0.08$	8.2	0.94	<0.001
Eurasia				
Gompertz	$K = 15977.18, M_0 = 25.14, \alpha = 0.05$	—	0.83	<0.001
Africa				
Gompertz	$K = 12900.31, M_0 = 0.44, \alpha = 0.06$	—	0.86	<0.001
North America				
Gompertz	$K = 6675.75, M_0 = 8.78, \alpha = 0.07$	—	0.85	<0.001
Carnivores				
Gompertz	$K = 710.56, M_0 = 14.62, \alpha = 0.10$	—	0.76	<0.001

The Gompertz model also provided good fits for the trajectories of maximum body size on each continent (Table 1 and fig. S2). Fifteen different lineages, representative of different archaic and modern orders (such as Proboscidea, Perissodactyla, Artiodactyla, Dinocerata, Pantodonta, Condylarthra, Xenarthra, etc.) evolved similar maximum size at different times and on different continents. These results show that the sigmoidal or saturating trajectory of maximal size evolution for Cenozoic mammals in North America (5, 8) occurred independently in multiple lineages on all the large continents. These results support the interpretation that similar niches were available to and filled by comparably sized giant mammals on each continent after 35 to 40 Ma. Because these niches were occupied by multiple different lineages at different times and on different continents, the patterns suggest that large mammals convergently evolved to fill similar ecological roles. Consistent with this idea, the largest mammals after the beginning of the Cenozoic were always herbivores. These patterns are also congruent with arguments relating the maximum body size of contemporary herbivorous mammals to constraints of diet and digestive physiology (16).

Carnivorous mammals showed similar saturating trajectories but attained smaller maximum sizes than coexisting mega-herbivores (Fig. 2). Large mammal-eating mammals were effectively absent in the early Paleocene; instead, birds, terrestrial crocodiles, snakes, and large lizards were the dominant carnivores (17). Once carnivorous mammal guilds began to diversify, however, they showed a similar trajectory to that of the herbivores—also well fit by a Gompertz function (Table 1). Although carnivores and herbivores started from a similar size immediately after the K/Pg, after ~30 million years the largest carnivores approached an asymptotic maximum about one order of magnitude smaller than that of the largest herbivores (Fig. 2). As with herbivores (Fig. 1A), the carnivores convergently evolved similar maximum sizes in different lineages: the archaic orders Creodonta and Mesonychia, and the modern order Carnivora. Although the duration of these clades overlapped, there was turnover in the ordinal affiliation of the largest carnivore, with each sequentially evolving to a maximum body mass of ~1000 kg (Fig. 2). After the initial size increase, the ratio of body masses of coexisting carnivorous and herbivorous mammals remained similar across the entire Cenozoic (Pearson correlation = 0.819, $P < 0.000$; fig. S4). This suggests at least an indirect relation in which the maximal sizes of carnivores followed the overall size distribution of mammals, but not necessarily a direct causal relation between the largest carnivores and herbivores. Indeed, the largest carnivores probably did not prey on the largest herbivores. The disparity in maximum size between carnivores and herbivores persists in contemporary mammals: Lions, tigers, and bears are about an order of magnitude smaller than elephants and rhinos. The asymptotic maximum size of carnivores of

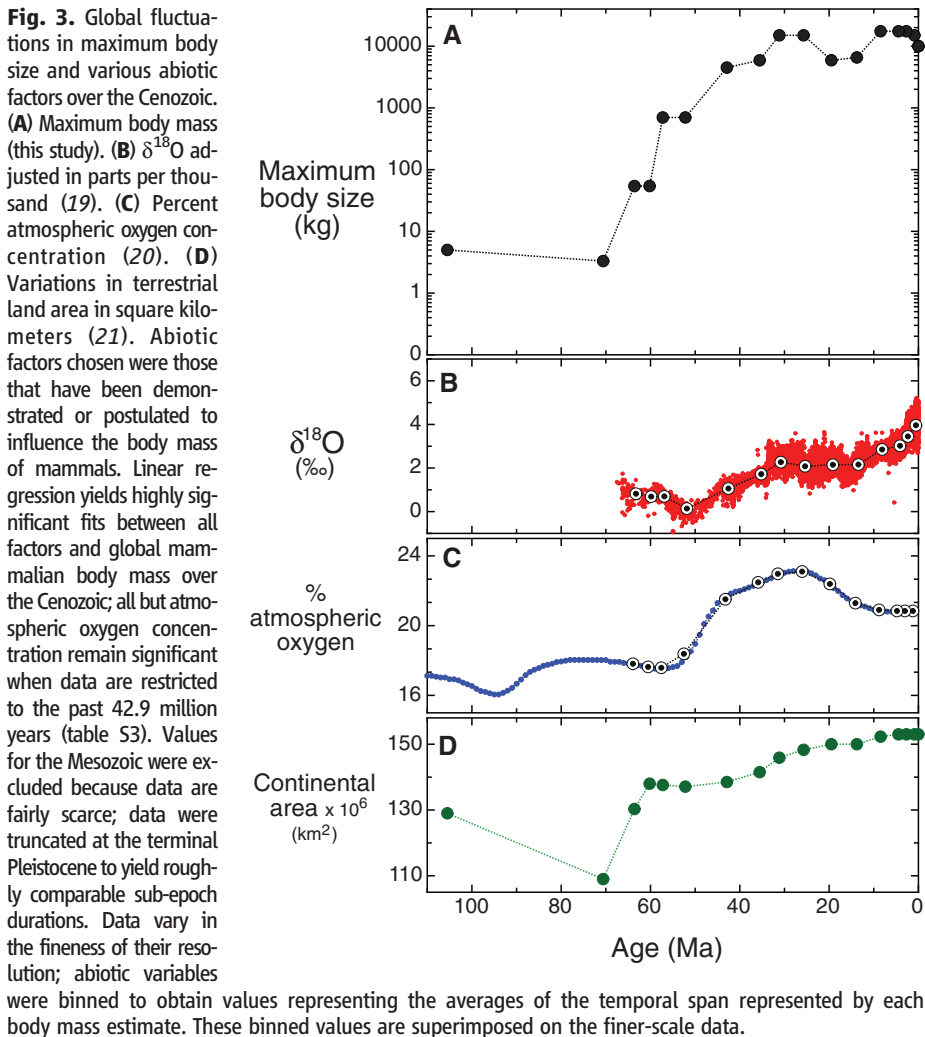
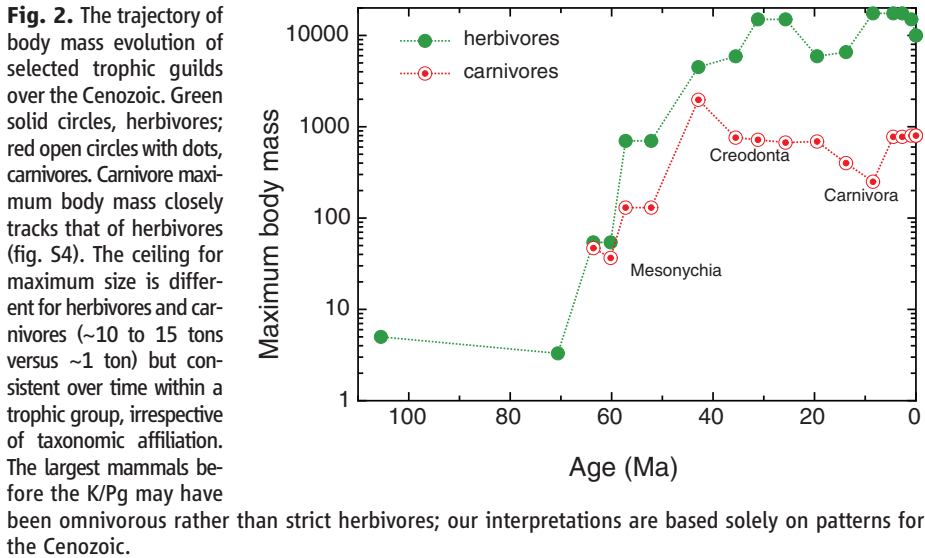
~1000 kg is consistent with the recent prediction that this represents an upper limit for flesh-eating terrestrial mammals because of physiological and ecological constraints (18).

We compared the overall global trajectory of maximum body mass with time series of three major abiotic factors: global temperature (19), atmospheric oxygen levels (20), and terrestrial

land area (21) (Fig. 3 and table S1). Each of these variables has been hypothesized theoretically and sometimes shown empirically to affect body size evolution in mammals: temperature by affecting how mammals dissipate heat through Bergmann's rule (22–24); greater land area by allowing larger populations and reducing extinction probabilities for the largest mammals (25, 26); and higher atmospheric oxygen concentrations by allowing higher rates of metabolism and biomass production (27–29). We averaged the abiotic values, which were generally reported at a finer scale, using the durations for each geological sub-epoch so we could compare against the trajectory of global body mass over the Cenozoic (table S1). Binned values are superimposed over the finer-scale data shown in Fig. 3. Our analyses were not based on specific values and slopes of these curves at specific times. We varied bin widths and averaging techniques; results were robust with regard to the binning technique employed (9). These abiotic records are based on proxies (19, 21) or on modeling of carbon isotopic records (20); hence, they contain significant unresolved uncertainties, which complicate interpretations of the patterns.

All abiotic factors were significantly related to mammalian body mass over the Cenozoic (Fig. 3 and table S3). To determine whether significance was driven by the initial exponential phase, we also ran analyses using the temporal interval from the late Eocene through the Pleistocene (42.9 to 0.9 Ma; results were similar when early or middle Eocene values were chosen). Both global temperature and terrestrial land area remained highly significant: The largest mammals evolved when Earth was cooler and terrestrial land area was greater (table S3), but atmospheric oxygen level dropped out (table S3). However, as might be expected, temperature and land area were significantly related (Pearson correlation = 0.904, $P < 0.001$, $df = 13$): Lower global environmental temperatures (indexed by $\delta^{18}\text{O}$) corresponded to more water stored in ice caps, lower sea levels, and increased land areas, and probably to changes in vegetation cover and primary productivity.

That temperature and/or land area may have influenced the evolution of body mass in mammals is consistent with several well-established biogeographic principles. The influence of temperature is consistent with Bergmann's rule, a well-known ecogeographic trend of larger body mass in cooler habitats across space (24), and in a few instances, across time (30). Bergmann's rule probably reflects physiological adaptations to prevent heat loss, because larger animals have a reduced surface-to-volume ratio; or alternatively, to promote heat dissipation at smaller body masses (24). Our results are also consistent with the hypothesis that available land area constrains the upper body mass limit of mammals by limiting population through the size or number of home ranges that can be "packed in" or by reducing energy acquisition (25, 26). Among contempo-



rary mammals, maximum body mass is strongly influenced by terrestrial land area, with larger-bodied mammals being found in larger insular or continental “islands” (fig. S5). Thus, constraints on maximum body size potentially imposed by both abiotic factors ultimately may be traced to physiological processes related to endothermy.

However, some caution should be used in the interpretation of our results. Quantitative analyses of these abiotic variables were complicated by a lack of resolution, potential collinearities, and a lack of statistical power that precluded the use of more-rigorous tests to fully explore the relationships between the predictor variables. Moreover, for some of these abiotic factors the uncertainties are not well characterized, and we currently have no way of knowing how these may interact to influence our results. For example, the oxygen isotope curve is confounded by changes in the terrestrial ice volume, atmospheric oxygen concentration is related to temperature through fluctuations in carbon dioxide and carbon sequestration (19) and potentially to global land area through changes in primary productivity, and global land area is clearly related to temperature and sea level. Moreover, other factors such as changes in seasonality and precipitation were not explicitly incorporated; the late Cenozoic saw a global trend toward cooler, drier, and more seasonal climates (19, 31). Nevertheless, the potential role of abiotic factors in the overall trajectory of mammalian evolution cannot be ignored, and the available data suggest interesting and important trends, which should be explored further.

Our analysis implies that the increase in the maximum mass of mammals over the Cenozoic was neither a statistical inevitability driven by increasing species richness nor a random evolutionary walk from a small initial size, but rather

reflected processes operating consistently across trophic and taxonomic groups, and independent of the physiographic history of each continent. We find no support for other hypotheses for the evolution of maximum body mass (9), including the expected increase in variance due to random divergence from a common ancestor or to increasing species richness (13–15); nor do terrestrial mammals ever approach sizes that might invoke biomechanical constraints (32). The K/Pg extinction provided the ecological opportunity for mammals to become larger. Terrestrial mammals did so in an exponentially decreasing fashion, reaching a more or less maximal size by 40 Ma as evolutionary possibilities for increasing body size were progressively exhausted and abiotic factors began constraining the upper limit.

References and Notes

1. Z.-X. Luo, *Nature* **450**, 1011 (2007).
2. A. W. Crompton, in *Comparative Physiology: Primitive Mammals*, K. Schmidt-Nielsen, L. Bolis, C. R. Taylor, Eds. (Cambridge Univ. Press, Cambridge, 1980), pp. 1–12.
3. Y. Hu, J. Meng, Y. Wang, C. Li, *Nature* **433**, 149 (2005).
4. Q. Ji, Z. X. Luo, C. X. Yuan, A. R. Tabrum, *Science* **311**, 1123 (2006).
5. J. Alroy, *Syst. Biol.* **48**, 107 (1999).
6. P. J. Van Soest, *The Nutritional Ecology of the Ruminant* (Cornell Univ. Press, ed. 2, Ithaca, NY, 1994).
7. J. A. Wolfe, G. R. Upchurch, *Nature* **324**, 148 (1986).
8. J. Alroy, *Science* **280**, 731 (1998).
9. Supporting material is available on Science Online.
10. A. W. Trites, D. Pauly, *Can. J. Zool.* **76**, 886 (1998).
11. F. M. Gradstein, J. G. Ogg, A. G. Smith, *A Geologic Time Scale 2004* (Cambridge Univ. Press, Cambridge, 2004).
12. www.paleodb.org/cgi-bin/bridge.pl
13. S. J. Gould, *J. Paleontol.* **62**, 319 (1988).
14. D. W. MacShea, *Evolution* **48**, 1747 (1994).
15. J. Trammer, *Evolution* **59**, 941 (2005).
16. M. Clauss et al., *Oecologia* **136**, 14 (2003).
17. G. F. Gunnell, M. E. Morgan, M. C. Maas, P. D. Gingerich, *Paleogeogr. Paleoclim. Paleoecon.* **115**, 265 (1995).
18. C. Carbone, A. Teacher, J. M. Rowcliffe, *PLoS Biol.* **5**, e22 (2007).
19. J. C. Zachos, G. R. Dickens, R. E. Zeebe, *Nature* **451**, 279 (2008).
20. P. G. Falkowski et al., *Science* **309**, 2202 (2005).
21. A. G. Smith, D. G. Smith, B. M. Funnell, *Atlas of Mesozoic and Cenozoic Coastlines* (Cambridge Univ. Press, Cambridge, 1994).
22. C. M. Janis, *Annu. Rev. Ecol. Syst.* **24**, 467 (1993).
23. G. F. Gunnell, in *Evolution of Tertiary Mammals of North America*, C. M. Janis, K. M. Scott, L. L. Jacobs, Eds. (Cambridge Univ. Press, Cambridge, 1998), pp. 91–109.
24. B. Rensch, *Evolution above the Species Level* (Columbia Univ. Press, New York, 1959).
25. P. A. Marquet, M. L. Taper, *Evol. Ecol.* **12**, 117 (1998).
26. G. P. Burness, J. Diamond, T. Flannery, *Proc. Natl. Acad. Sci. U.S.A.* **98**, 14518 (2001).
27. H. Tappan, in *Molecular Oxygen in Biology: Topics in Molecular Oxygen Research*, O. Hayaishi, Ed. (North-Holland, Amsterdam, 1974), pp. 81–135.
28. J. L. Payne et al., *Proc. Natl. Acad. Sci. U.S.A.* **106**, 24 (2009).
29. R. H. Peters, *The Ecological Implications of Body Size* (Cambridge Univ. Press, Cambridge, 1983).
30. F. A. Smith, J. L. Betancourt, J. H. Brown, *Science* **270**, 1202 (1995).
31. G. Retallack, *J. Geol.* **109**, 407 (2001).
32. R. M. Alexander, *Paleontology* **4**, 1231 (1998).
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Modular Organic Structure-Directing Agents for the Synthesis of Zeolites

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Organic structure-directing agents (OSDAs) are used to guide the formation of particular types of pores and channels during the synthesis of zeolites. We report that the use of highly versatile OSDAs based on phosphazenes has been successfully introduced for the synthesis of zeolites. This approach has made possible the synthesis of the elusive bogsite zeolite, which is formed by 10- and 12-ring intersecting channels. This topology and these pore dimensions present interesting opportunities for catalysis in reactions of industrial relevance.

Zeolites are crystalline microporous and mesoporous materials (1–4) that offer a wide range of applications because of their well-defined structures, which are formed by channels with pore apertures of molecular dimensions. An important objective during the synthesis of zeolites is to achieve control of the pore dimensions and their connectivity through the use of organic structure-directing agents (OSDAs) that, at the

limit, could act as template molecules. A large variety of quaternary organic ammonium salts have been successfully used as OSDAs (2, 4–6) as well as analogous molecules, such as phosphonium-derived organic cations (7–10). However, rather than design new molecules for each zeolite target it could be more efficient to have a type of OSDA that could be easily built by blocks similar to Legos, with a large variety of substituents. Poten-

tial new structures could be simulated with molecular modeling techniques, and an OSDA that directs its synthesis by minimizing the energy of the zeolite-OSDA system could be predicted or at least can be selected from a limited number of candidates.

The described procedure requires having a tool box of OSDA molecules that are easy to prepare and adapt while having the adequate polarity and basicity. We present a type of OSDA molecule with a nearly unlimited synthesis flexibility that is based on building-block units. These molecules are based on phosphazenes that can mobilize silica, have the adequate polarity and stability, and offer more structural possibilities than quaternary ammonium or phosphonium cations. We used these OSDAs for the synthesis of new zeolite structures,

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including boggsite, which is a rare, naturally occurring zeolite that has pores of different dimensions and that to date has proven difficult to synthesize. Such targets are important because zeolites with pores of differently sized apertures within the same structure may have catalytic properties that arise when reactants and/or products of different molecular dimensions could react and diffuse (11). In this sense, the synthesis of zeolites with high Si/Al ratios and intersecting channels having pore apertures delimited by 10 and 12 tetrahedra (10-ring and 12-ring pores) should be of much interest because of the combination of selectivity and stability. A preferred type of structure should have discrete channels formed by 10 and 12 rings, both connected to the exterior, which are better than 12-ring channels connected by 10-ring windows or vice versa. For instance, the synthetic zeolite NU-87 (NES) (12) was synthesized, but the access to the inner part of the crystal can only occur through the 10-ring pores.

Three additional molecular sieves [CIT-1, SSZ-26, and SSZ-33 (CON)] with intersecting 10- and 12-ring pores were prepared (13–16). The SSZ-26 and SSZ-33 zeolites are an intergrowth of two polymorphs, “A” and “B,” with a predominance of polymorph B (13), whereas CIT-1 is the end-member corresponding to pure polymorph B of said intergrowth (16). Molecular simulations revealed that the OSDA for SSZ-26 is a good match to the geometry at the intersections of 10- and 12-ring pores (4). However, the 10-ring system of SSZ-26 and SSZ-33 does not correspond to pores but to windows. More recently, another polymorph of the above family [polymorph C, ITQ-27 (IWR)] was synthesized (17), but again its pore system can be better considered as formed by 12-ring channels connected by 10-ring windows. There is, however, a natural zeolite named boggsite (BOG) (18) that has intersecting 10- and 12-ring pores, both being open to the external surface. This is a rare mineral that occurs in minute quantities at Goble, Columbia County, Oregon (northwest United States) (18) and at Mt. Adamson, Northern Victoria Land (Antarctica) (19). The naturally occurring material has a Si/Al ratio close to 4.2.

In an attempt to synthesize boggsite, and taking into account that the framework of some of the already existing 10-ring-by-12-ring molecular sieves contain the 4–4=1 secondary building unit (20), which is also present in boggsite, we performed a computer simulation in an attempt to see whether any of the reported OSDAs for zeolites with intersecting 10- and 12-ring pores could stabilize the structure of boggsite. Then, the OSDAs reported for the synthesis of CIT-1 (CON) (16), ITQ-24 (IWR) (17), ITQ-22 (IWW) (21), MCM-68 (MSE) (22), SSZ-56 (SFS) (23), and IM-6 (USI) (24) were studied with an atomistic force field in order to calculate the minimum energy of the OSDAs within the microporous space of a pure silica boggsite (table S1) (25). The results in table S1 show the stabilization energy ($E_{\text{ZeO-OSDA}}$) of these different OSDAs for boggsite. OSDA-CON, OSDA-SFS, and OSDA-USI have energies near

4.3 kJ/mol and could help stabilize the structure of boggsite. However, when we carried out the syntheses with some of these OSDAs we could not obtain boggsite.

We then tried an OSDA based on uncharged superbasic phosphazenes (26). These molecules (Fig. 1) can mobilize silica in aqueous solution because their high basicities allow them to react with water, yielding to the corresponding phosphazanium hydroxides (Fig. 2). They have an adequate polarity and allow more structural possibilities than quaternary ammonium or phosphonium OSDAs because of the large number of alkyl (R) groups and the nearly unlimited synthesis flexibility that is based on building blocks. Thus, a series of phosphazenes, some of which are commercially available, were considered as potential OSDAs (table S1).

The stabilization of BOG structure was studied with those OSDAs, and results presented in table S1 show that with the commercial tert-butyl-imino-tris(dimethylamino)phosphorane (t-Bu-P1) (fig. S1), the best stabilization of boggsite was achieved. Indeed, one of the first attempts to synthesize boggsite by using t-Bu-P1 as OSDA produced the BOG structure as a minor phase of a complex mixture of at least three different materials. Then, a high-throughput synthesis design of experiments was established, and after determining the most important synthesis variables (fig. S2) the material ITQ-47 was obtained that has an x-ray diffraction (XRD) pattern nearly the same as that

reported for the mineral boggsite (27). The structure of ITQ-47 was confirmed through XRD to be identical topologically to the boggsite mineral, differing from the mineral in the chemical composition, because the zeolite ITQ-47 contains B whereas the natural boggsite occurs as Al-silicate. Indeed, the XRD pattern of the calcined ITQ-47 material was indexed according to an orthorhombic unit cell with the following unit cell parameters: $a = 20.014$, $b = 23.580$, and $c = 12.669$ Å. The Rietveld refinement of the crystal structure was successfully performed in the space group *Imma*. The refined atomic coordinates are shown in table S3, and Fig. 3 shows the good agreement between the observed and calculated XRD patterns. The details of the Rietveld refinement are given in (25).

The projections of ITQ-47 structure along the different crystallographic axes are shown in Fig. 4. There, the presence of a bidirectional pore system with intercrossing 12- and 10-ring channels is shown. The first set of 12-ring straight channels runs perpendicularly to the bc plane, with a nearly circular pore aperture of approximately 7.0 Å diameter. A second set of 10-ring straight channels is placed along the b axis with a pore aperture of 5.5 by 5.3 Å. The channels intercross one to each other, giving rise to the formation of relatively large void spaces inside of the boggsite structure. Lastly, there are no pores large enough to allow access of molecules inside boggsite structure along the c axis.

Fig. 1. Examples of phosphazene structures.

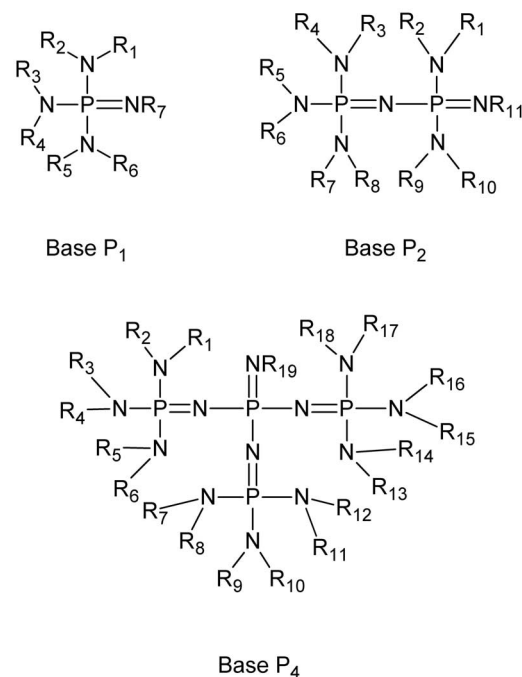
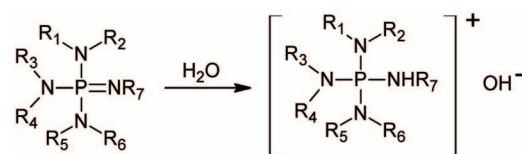


Fig. 2. Scheme of formation of corresponding phosphazanium hydroxide.



The presence of pores of different sizes within the same structure can be seen in the micropore distribution calculated by applying the Horvath-Kawazoe formalism to the Ar adsorption isotherm of the calcined ITQ-47 material (fig. S3). There, the presence of a maximum at 6.4 Å was observed, which corresponds to the 12-ring channels along the *a* axis; this maximum is accompanied by a shoulder at approximately 5.4 Å assigned to the pore aperture of 10-ring channels running along the *b* axis. The total micropore volume (calculated from the N₂ adsorption isotherm by applying the *t*-plot method) and BET surface area were 0.22 cm³/g and 458 m²/g, respectively, which are also consistent with the void space present in the bogg site structure.

These organic molecules remain intact and fill the pores and cavities of the zeolites upon crystallization, as has been confirmed by means of ³¹P and ¹³C-magic angle spinning-nuclear magnetic resonance (MAS-NMR) spectroscopy and chemical analyses. Indeed, the characteristic ³¹P resonance of P1-t-Bu phosphazene at 34 parts per million (ppm) is the only signal present in the ³¹P-MAS-NMR spectrum of the as-made ITQ-47

material. Moreover, the signals appearing in the ¹³C-cross-polarization (CP)/MAS-NMR spectrum of the as-made ITQ-47 material closely correspond to those observed for the liquid ¹³C-CP-NMR spectrum of P1-t-Bu in D₂O (fig. S4). The chemical composition of a well-washed sample (25) indicates that practically no incorporation of Ge occurs during the synthesis, being the framework formed by Si and B. Furthermore, the chemical and elemental analyses of the as-made ITQ-47 material (table S4) give P:N and N:C ratios near those expected for pure P1-t-Bu. Thus, P1-t-Bu does not decompose during bogg site crystallization and remains inside the channels filling the pores. The B:P ratio is nearly one, which strongly suggests that besides filling the pores, P1-t-Bu moieties are compensating the framework negative charge caused by the isomorphous substitution of B by Si. The isomorphous incorporation of B is further supported by the presence of a resonance at -2 ppm in the ¹¹B-MAS-NMR spectrum of the as-made sample (fig. S5).

The calcined B-ITQ-47 material was also studied through multi-nuclei MAS-NMR techniques. First, ¹¹B-MAS-NMR spectrum of the cal-

cined B-ITQ-47 solid shows the typical line shape of a mixture of tetrahedral and trigonal B species, which are found in the partially hydrated zeolites (28). All B species revert to a fully tetrahedral environment after washing the sample (fig. S5), as it has been seen in B-containing zeolites upon full hydration (28, 29).

The presence of P debris species formed upon calcination of ITQ-47 was confirmed through ³¹P-MAS-NMR spectroscopy. Indeed, the presence of a number of resonances in the range of 0 to -50 ppm indicates the formation of a complex mixture of phosphate polyanions with different degrees of polymerization. However, these phosphate-like species can be easily removed by washing the calcined ITQ-47 with an ammonium acetate aqueous solution, which was confirmed by the absence of relevant signals in the ³¹P-MAS-NMR spectrum of the washed ITQ-47 material as well as with chemical analyses (fig. S6 and table S4).

The main goal of synthesizing bogg site was to study its acid and catalytic properties, but B-ITQ-47 shows very weak acidity, precluding its catalytic use for many processes. We exchanged Al(III) for B(III) (30) in order to increase the acidity of the material. When this is done, Al-ITQ-47 is obtained, which develops strong acidity able to retain pyridine even at 623 K under vacuum (fig. S7). The isomorphous incorporation of Al atoms in the ITQ-47 zeolite was confirmed with ²⁷Al-MAS-NMR spectroscopy (fig. S9). The spectrum of the Al-exchanged sample shows an intense resonance at 55 ppm, which is attributed to tetrahedrally coordinated Al centers in silica environments and is taken as evidence of the incorporation of Al atoms in zeolite frameworks. This main signal is accompanied by a minor and narrow resonance at 0 ppm because of the presence of a very minor proportion of Al in extraframework positions.

The Al-exchanged samples are active for acid-catalyzed reactions, such as cracking, isomerization, and dealkylation-transalkylation of aromatics. The bogg site structure should also be particularly appropriated for aromatic alkylations, owing to the interconnected 12- and 10-ring channels. This pore topology and pore dimensions may be

Fig. 3. Rietveld refinement of ITQ-47 zeolite after calcination and subsequent washing. Observed (circles) and calculated (line) XRPD patterns, and (bottom) difference profile. The short tick marks below the pattern give the positions of the Bragg reflections. arb. units, arbitrary units.

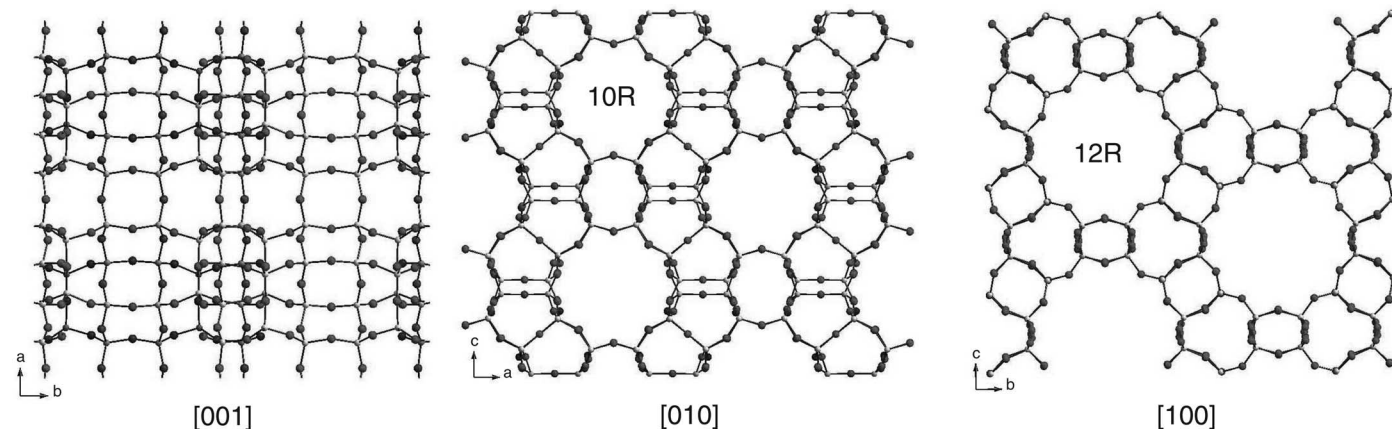
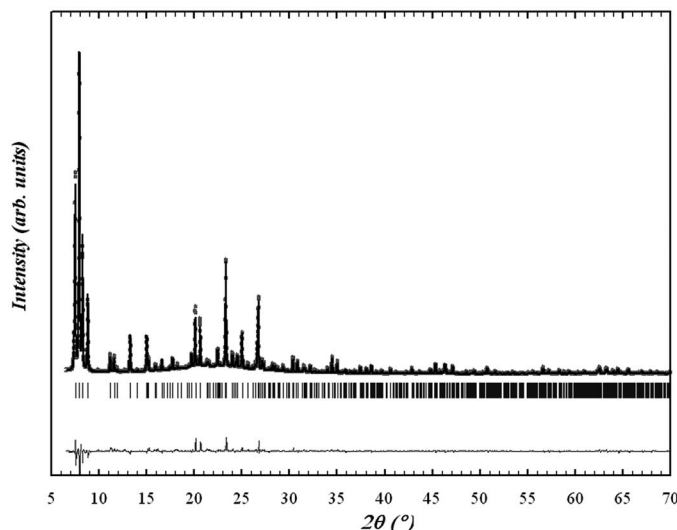


Fig. 4. Structure of zeolite ITQ-47 viewed along the different crystallographic axes.

adequate for the industrially relevant synthesis of cumene through the alkylation of benzene with propylene because it should give lower yields of undesired secondary products when compared with the commercially used 12-ring beta zeolite. We have checked this possibility and analyzed the results of selectivity versus conversion for cumene/propylene alkylation on Al-ITQ-47 and beta (fig. S10). Regardless of the level of conversion considered, the selectivity to cumene is always greater for Al-ITQ-47 with boggsite structure than for beta zeolite. However, the activity of beta zeolite is greater than Al-ITQ-47 because of the smaller crystal size of beta zeolite than that of boggsite (fig. S11).

References and Notes

- J. Jiang, J. Yu, A. Corma, *Angew. Chem. Int. Ed.* **49**, 3120 (2010).
- A. W. Burton, S. I. Zones, S. Elomari, *Curr. Opin. Colloid Interface Sci.* **10**, 211 (2005).
- J. Sun et al., *Nature* **458**, 1154 (2009).
- R. F. Lobo, S. I. Zones, M. E. Davis, *J. Inclusion Phenom. Mol. Recognit. Chem.* **21**, 47 (1995).
- A. Jackowski, S. I. Zones, S.-J. Hwang, A. W. Burton, *J. Am. Chem. Soc.* **131**, 1092 (2009).
- R. H. Archer, S. I. Zones, M. E. Davis, *Microporous Mesoporous Mater.* **130**, 255 (2010).
- D. L. Dorset et al., *J. Am. Chem. Soc.* **128**, 8862 (2006).
- A. Corma et al., *J. Am. Chem. Soc.* **130**, 16482 (2008).
- D. L. Dorset et al., *Chem. Mater.* **20**, 5325 (2008).
- A. Corma et al., *Proc. Natl. Acad. Sci. U.S.A.* **107**, 11935 (2010).
- A. Corma, *Microporous Mesoporous Mater.* **21**, 487 (1998).
- M. D. Shannon, J. L. Casci, P. A. Cox, S. J. Andrews, *Nature* **353**, 417 (1995).
- R. F. Lobo et al., *Science* **3**, 1543 (1993).
- S. I. Zones, M. M. Olmsted, D. S. Santilli, *J. Am. Chem. Soc.* **114**, 4195 (1992).
- S. I. Zones et al., U.S. Patent 491006 (1990).
- R. F. Lobo, M. E. Davis, *J. Am. Chem. Soc.* **117**, 3766 (1995).
- R. Castañeda, A. Corma, V. Fornés, F. Rey, J. Rius, *J. Am. Chem. Soc.* **125**, 7820 (2003).
- J. J. Pluth, J. S. Smith, *Am. Mineral.* **75**, 501 (1990).
- E. Galli, S. Quartieri, G. Vezzadini, A. Alberti, *Eur. J. Mineral.* **7**, 1029 (1995).
- Ch. Baerlocher, L. B. McCusker, D. H. Olson, *Atlas of Zeolite Framework Types* (Elsevier, Amsterdam, ed. 6, 2007).
- A. Corma, F. Rey, S. Valencia, J. L. Jordá, J. Rius, *Nat. Mater.* **2**, 493 (2003).
- D. L. Dorset, S. C. Weston, S. S. Dhingra, *J. Phys. Chem. B* **110**, 2045 (2006).
- S. Elomari, A. Burtin, R. C. Medrud, R. Grosse-Kunstleve, *Microporous Mesoporous Mater.* **118**, 325 (2009).
- L. Josien, A. Simon-Masseron, V. Gramlich, J. Patarin, L. Rouleau, *Chemistry* **9**, 856 (2003).
- Materials and methods are available as supporting material on Science Online.
- R. Schwesinger et al., *Liebigs Ann.* **7**, 1055 (1996).
- D. G. Howard, R. W. Tschernich, J. V. Smith, G. L. Klein, *Am. Mineral.* **75**, 1200 (1990).
- C. Fild, D. F. Shantz, R. F. Lobo, H. Koller, *Phys. Chem. Chem. Phys.* **2**, 3091 (2000).
- R. H. Koller, C. Fild, R. F. Lobo, *Microporous Mesoporous Mater.* **79**, 215 (2005).
- C. Chen, S. I. Zones, *Stud. Surf. Sci. Catal.* **135**, 1710 (2001).
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Renewable Chemical Commodity Feedstocks from Integrated Catalytic Processing of Pyrolysis Oils

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Fast pyrolysis of lignocellulosic biomass produces a renewable liquid fuel called pyrolysis oil that is the cheapest liquid fuel produced from biomass today. Here we show that pyrolysis oils can be converted into industrial commodity chemical feedstocks using an integrated catalytic approach that combines hydroprocessing with zeolite catalysis. The hydroprocessing increases the intrinsic hydrogen content of the pyrolysis oil, producing polyols and alcohols. The zeolite catalyst then converts these hydrogenated products into light olefins and aromatic hydrocarbons in a yield as much as three times higher than that produced with the pure pyrolysis oil. The yield of aromatic hydrocarbons and light olefins from the biomass conversion over zeolite is proportional to the intrinsic amount of hydrogen added to the biomass feedstock during hydroprocessing. The total product yield can be adjusted depending on market values of the chemical feedstocks and the relative prices of the hydrogen and biomass.

Dwindling petroleum resources combined with economic, environmental, and political concerns about the petroleum-based economy make it imperative to develop new processes for the production of renewable fuels and chemicals (1–3). Lignocellulosic biomass is the most abundant and inexpensive sustainable source of carbon that can be used as a

feedstock for the production of renewable fuels and commodity chemical feedstocks (4). Pyrolysis oils or bio-oils have been identified as inexpensive renewable liquid fuel that can be produced from lignocellulosic biomass (5–7). Fast pyrolysis of biomass has been shown to be two to three times cheaper than biomass conversion technologies based on gasification and fermentation processes (8). However, bio-oils are low-quality fuels that cannot be used in conventional gasoline and diesel fuel engines because they are immiscible with petroleum-derived fuels, primarily on account of their high oxygen content [up to 60 weight % (wt %)] (5, 7, 9). Other challenges with pyrolysis oils are that

they are acidic, have a high water content (25 to 50 wt %), and constitute an emulsion that will phase-separate when stored. Ideally, pyrolysis oils should be deoxygenated to a mixture of organic molecules that are more compatible with current fuels and chemical manufacturing infrastructure (10). Several approaches toward this end are currently being studied, including hydrotreating of bio-oils (10, 11), zeolite conversion of bio-oils (12, 13), and aqueous-phase processing of bio-oils (14). However, none of these approaches are used commercially today, primarily because they produce low yields of fungible products.

In this paper, we outline a distinct strategy for bio-oil deoxygenation into high-yield commodity chemicals, including C2 to C6 monohydric alcohols and diols, C6 to C8 aromatic hydrocarbons, and C2 to C4 olefins with over 60% overall carbon yields. Our approach involves hydroprocessing of the bio-oils over supported metal catalysts, followed by conversion over zeolite catalysts. It is not possible to completely hydrodeoxygenate the pyrolysis oil in a packed-bed reactor without frequent catalyst regeneration because of coke formation on the catalyst surface. Furthermore, complete hydrodeoxygenation requires large amounts of expensive hydrogen. In our process, drawbacks associated with the prior bio-oil hydrogenation processes are overcome by operating at moderate temperatures ($\leq 250^\circ\text{C}$) at which no catalyst coking or reactor plugging was observed. Furthermore, our process can produce products without the high hydrogen requirements. Employing a zeolite upgrading step at the end has the advantage that a fluidized bed reactor can be used, where the coked catalyst can be regenerated by burning off the coke and recy-

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cluded back to the reactor. The goal of this report is to demonstrate how pyrolysis oil could practically be upgraded through catalytic processes into commodity chemicals.

The integrated catalytic approach presented in this report can be tuned to produce different targeted distributions of organic small molecules that fit seamlessly into the existing petrochem-

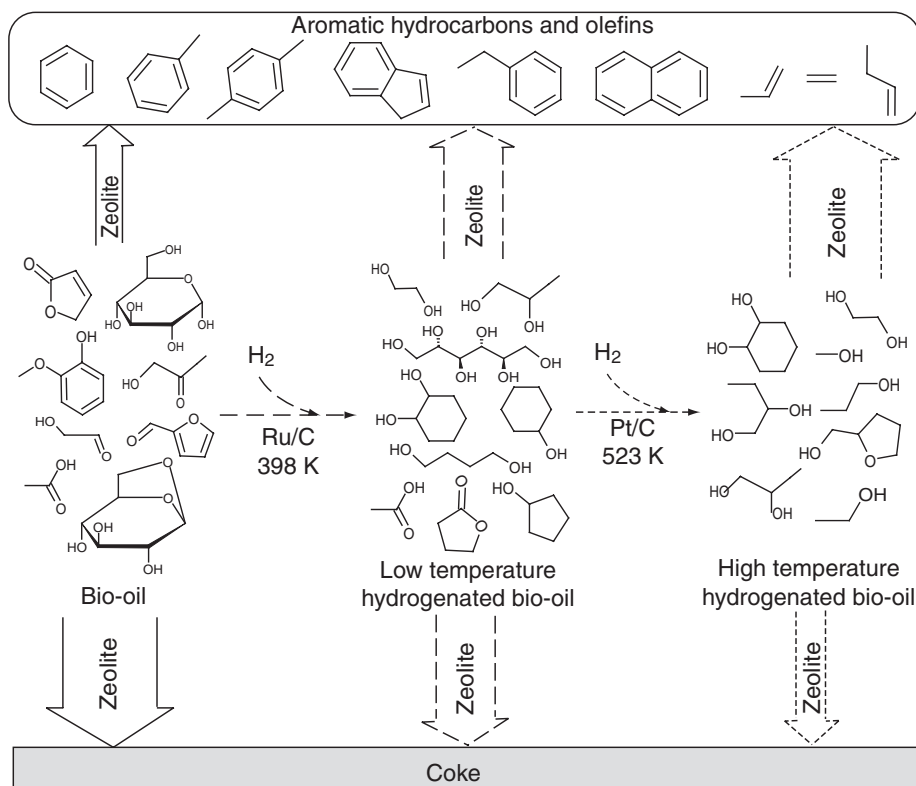
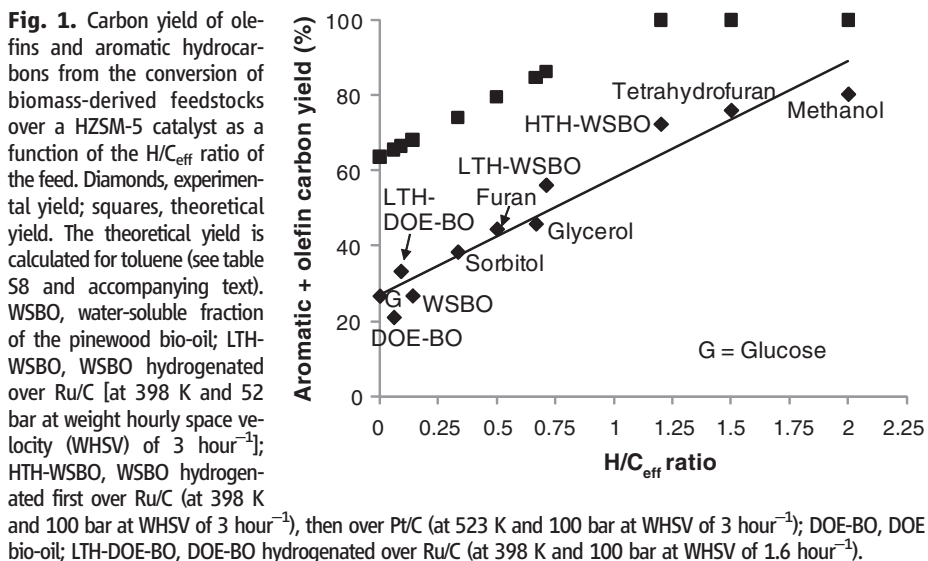


Fig. 2. Reaction schematic for the integrated hydroprocessing and zeolite upgrading of pyrolysis oil. Solid arrows: Pyrolysis oil is directly passed over the zeolite catalyst; long-dashed arrows: Pyrolysis oil is hydrogenated over Ru/C at 398 K and then passed over the zeolite catalyst; short-dashed arrows: Pyrolysis oil is first hydrogenated over Ru/C at 398 K, then over Pt/C at 523 K, and then passed over the zeolite catalyst. The width of the vertical arrows represents the product carbon yield from a particular feed. In addition to the product streams shown in the figure, oxygen is removed at the zeolite stage as a mixture of CO, CO₂, and H₂O. Boosting the hydrogen content of the zeolite feed (left to right) increases the thermal stability of the feed, resulting in a reduction in amount of coke and an increase in the yields of aromatic hydrocarbons and olefins. The addition of hydrogen also raises the proportion of oxygen lost as water relative to CO and CO₂ and thereby further raises the proportion of carbon incorporated into marketable compounds.

ical infrastructure. The products can be tuned to change with different market conditions. The C6 to C8 aromatic hydrocarbons can be high-octane gasoline additives or feedstocks for the chemical and polymer industries (15). The C2 to C4 olefins can also be used directly for polymer synthesis or can be modified to form other products, including alkylated aromatics (16, 17) and longer linear alpha olefins (18). The gasoline-range alcohols can be high-octane gasoline additives. The C2 to C6 diols can serve as feedstocks for the chemical and polymer industries. The chemical industry relies on seven primary building blocks that are all derived from petroleum-based processes: benzene, toluene, xylene, ethylene, propylene, 1,3-butadiene, and methanol (19). Our catalytic process produces five of these seven petrochemical feedstocks, which opens the door to a chemical industry based on renewable biomass feedstock.

The hydrogen content of a particular feedstock can be expressed in terms of its hydrogen-to-carbon atomic effective ratio (H/C_{eff} ratio), as defined in Eq. 1. The H/C_{eff} ratio is equal to the number of atoms of hydrogen minus twice the number of atoms of oxygen divided by the number of atoms of carbon in a feedstock. Lignocellulosic biomass and carbohydrate-based feedstocks have H/C_{eff} ratios between 0 and 0.5. In contrast, petroleum-based feedstocks have H/C_{eff} ratios from 1.0 to 2.0. Thus, biomass-based feedstocks are hydrogen-deficient because of the presence of oxygen. For example, the bio-oil used in this study has an H/C_{eff} ratio of 0.06. During the conversion of biomass to deoxygenated compounds, the oxygen can be removed as a combination of CO, CO₂, and H₂O. Adding external hydrogen to supplement the hydrogen in the biomass itself boosts the proportion of oxygen removed as H₂O versus CO and CO₂. The exact ratios of CO, CO₂, and H₂O can be determined from the reaction stoichiometry between the feeds and the products. Thus, the addition of external hydrogen can increase the carbon yield of commodity chemicals produced from pyrolysis oils. This relation demonstrates how the biomass-refining industry could be tied to the hydrogen economy.

$$H/C_{\text{eff}} = \frac{\text{moles of H} - (2 \times \text{moles of O})}{\text{moles of C}} \quad (1)$$

In this study, we converted 11 different biomass-derived feedstocks, with a range of different functionalities over a ZSM-5 catalyst. This catalyst has the proper pore structure and active sites to effectively convert biomass-derived molecules into aromatic hydrocarbons and olefins (20–22). Figure 1 shows that the aromatic and olefin yield increases with the H/C_{eff} ratio of the feedstock (details in table S5), and this relationship is applicable to a wide variety of biomass-derived feedstocks. The theoretical yield (table S8 and accompanying text), also shown

in Fig. 1, increases with the H/C_{eff} ratio as well. The increase in the aromatic and olefin yield with increasing H/C_{eff} ratio is due to two different phenomena: an increase in the thermal stability of the feedstock and an increase in the intrinsic amount of hydrogen in the feedstock. Increasing the H/C_{eff} ratio improves the thermal stability of the biomass-derived molecules by hydrogenating the functionalities (primarily aldehydes and ketones) that otherwise thermally decompose to char as shown in table S7. For example, in thermogravimetric studies, glucose produces 23.1 wt % coke when heated to 973 K under a helium atmosphere (table S7). Sorbitol, a glucose derivative in which the aldehyde functionality has been hydrogenated to an alcohol, is substantially more thermally stable and produces only 6.1 wt % coke. Increasing the H/C_{eff} ratio also increases the intrinsic hydrogen content of the biomass-derived feedstock, which allows a higher theoretical yield of aromatic hydrocarbons and olefins from these feedstocks, because less carbon is used for the deoxygenation (table S8).

In this same respect, bio-oil and its aqueous fraction can be stabilized by hydroprocessing, and at the same time an increase in intrinsic hydrogen content results in higher aromatic and olefin yields. Figure 2 shows a generic reaction scheme for the conversion of bio-oil (or the water-soluble fraction of bio-oil) by hydroprocessing and zeolite upgrading to various products. The thermally unstable carbonyl functionalities in bio-oil go directly to coke on the zeolite catalyst, bypassing the desired commodity chemicals, including aromatic hydrocarbons, olefins, and alcohols. The carbonyl functionalities are converted to thermally stable corresponding alcohols upon hydrogenation, and the coke yield decreases in zeolite upgrading. A second hydrogenation step further increases the H/C_{eff} ratio of feed, resulting in even lower coke yields in zeolite upgrading.

Figure S1 shows different process options we used in this study for the conversion of pyrolysis oil. The crude pyrolysis oil can be subjected directly to our process, or it can first be phase-separated into an aqueous and an organic phase by the addition of water. The organic phase of the bio-oil mainly contains high-molecular-weight lignin-derived phenolic oligomers (23). These lignin oligomers can be used to make phenol-formaldehyde resins (24) or as a low-cost feedstock for the base catalyzed depolymerization processes (25–27) to produce phenolic compounds or aromatic fuel (28–30). To exploit the advantages of hydroprocessing and zeolite upgrading for the upgrading of pyrolysis oil, we opted to study three different processing steps that can be operated in series: a low-temperature hydrogenation step using a Ru-based catalyst, a higher-temperature hydrogenation step using a Pt-based catalyst after the Ru-based step, and a zeolite conversion step. The Ru catalyst was chosen based on our previous experimental and theoretical studies on aqueous-phase acetic acid

hydrogenation over monometallic catalysts (31). The conclusion from this study was that Ru was the most active and selective monometallic catalyst for aqueous-phase acetic acid hydrogenation at low temperatures. Pt was chosen as the catalyst for the high-temperature hydrogenation, because previous studies for hydrodeoxygenation of sorbitol have indicated that Pt catalysts have high C–O hydrogenation and low C–C bond cleavage activity (32). We explored these conversion options with two different pyrolysis oil feeds: crude bio-oil obtained from the U.S. Department of Energy's National Renewable Energy Laboratory (DOE-BO), and the water-soluble fraction of a pinewood bio-oil (WSBO). The WSBO sample was made by mixing pinewood bio-oil and water in a 1:4 weight ratio (33). For the DOE-BO sample, we compared the outcomes of zeolite upgrading with and without preceding low-temperature hy-

drogenation. For the WSBO sample, we examined these two protocols as well as the third, incorporating a high-temperature hydrogenation step between the low-temperature hydrogenation and zeolite upgrading steps.

Gas chromatography (GC) and high-performance liquid chromatography (HPLC) of the DOE-BO (table S1) identified hydroxyacetaldehyde (9.0% of the total bio-oil carbon content), levoglucosan (8.8% of total bio-oil carbon), acetic acid (8.4% of total bio-oil carbon), sugars (1.8% of total bio-oil carbon), and hydroxyacetone (1.6% of total bio-oil carbon) as major components. Only one-third of the carbon content quantified by elemental analysis could be identified with these techniques. The remaining two-thirds of the carbon can be attributed to nonquantified compounds that are present in small quantities and GC- and HPLC-undetectable lignin and sugar oligomers. Gel per-

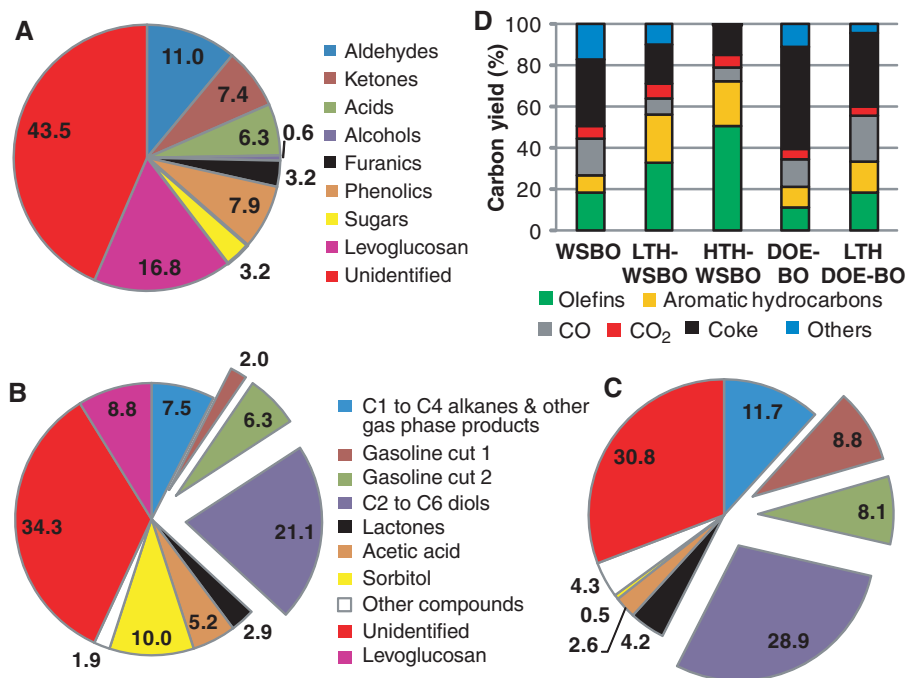


Fig. 3. Feed and product carbon distribution (in percent of carbon) for the hydrogenation of WSBO and for the zeolite upgrading. (A) WSBO feed. (B) Product distribution from single-stage hydrogenation of WSBO over Ru/C at 398 K and 52 bar at WHSV of 3 hour⁻¹. (C) Product distribution from two-stage hydrogenation of WSBO, first over Ru/C at 398 K and 100 bar at WHSV of 3 hour⁻¹, then over Pt/C at 523 K and 100 bar at WHSV of 3 hour⁻¹. (D) Carbon yields for the conversion of bio-oil-derived feedstocks over HZSM-5 at 873 K. WSBO, water-soluble fraction of the pinewood bio-oil; LTH-WSBO, WSBO hydrogenated over Ru/C (at 398 K and 52 bar at WHSV of 3 hour⁻¹); HTH-WSBO, WSBO hydrogenated first over Ru/C (at 398 K and 100 bar at WHSV of 3 hour⁻¹), then over Pt/C (at 523 K and 100 bar at WHSV of 3 hour⁻¹); DOE-BO, DOE bio-oil; LTH-DOE-BO, DOE bio-oil hydrogenated over Ru/C (at 398 K and 100 bar at WHSV of 1.6 hour⁻¹). The category "others" encompasses phenol, alkyl phenols, naphthol, and alkyl naphthols. The product groups defined in (B) and (C) contain the following components: C1 to C4 alkanes and other gas-phase compounds: methane, ethane, propane, butane, and other gas-phase compounds that have not been identified. Gasoline cut 1 (boiling range 338 to 373 K): pentane, hexane, methanol, ethanol, 1-propanol, tetrahydrofuran, 2-butanol, and 2-methyltetrahydrofuran. Gasoline cut 2 (boiling range 388 to 448 K): 1,2-cyclohexanediol, 2,5-dimethyltetrahydrofuran, 1-butanol, 2-pentanol, 1-pentanol, cyclopentanol, 2-hexanol, 3-methylcyclopentanol, cyclohexanol, 3-methylcyclohexanol, and 4-methylcyclohexanol. C2 to C6 diols: 2,3-butanediol, propylene glycol, ethylene glycol, 1,2-hexanediol, 1,4-hexanediol, 1,4-butanediol, and 1,4-pentanediol. Lactones: γ -butyrolactone and γ -valerolactone. Other compounds: tetrahydrofurfuryl alcohol, 1,2,6-hexanetriol, 1,2,3-butanetriol, and glycerol.

meation chromatography (GPC) (fig. S2) of DOE-BO confirmed the presence of oligomers ranging in molecular weight from 300 to 7000 daltons. Analysis of the WSBO by GC and HPLC identified the compounds composing 56.5% of the total carbon content (Fig. 3A and table S3). The most abundant functionalities in the WSBO included carbonyl compounds (24.7% of total WSBO carbon), carbohydrates (20% of total WSBO carbon; includes sugars and levoglucosan), and phenolics (7.9% of total WSBO carbon). The WSBO had an H/C_{eff} ratio of 0.14. The unidentified carbon in the WSBO is most likely present as undetectable lignin and sugar oligomers.

We next examined the composition of the product stream from low-temperature hydrogenation of the DOE-BO. The H/C_{eff} ratio rose from 0.06 to 0.09, with about 0.9 g of hydrogen consumed per 100 g of carbon in the feed during this step. The water content of the bio-oil also increased from 24.8 to 27 wt % during the low-temperature hydrogenation step. At the same time, the proportion of carbon content identifiable by GC and HPLC went down (table S2), perhaps as a result of homogeneous thermal po-

lymerization reactions among the bio-oil components. The GPC analysis of the hydrogenated DOE bio-oil (fig. S2) shows higher concentrations of oligomers with molecular weights greater than 400 daltons as compared to the feed. The amount of thermal coke formed from DOE-BO was reduced from 19.0 to 13.6 wt % upon low-temperature hydrogenation (table S7). This implies that the product of low-temperature hydrogenation of DOE-BO is more thermally stable than the feed.

In the case of the water-soluble fraction of the WSBO, the H/C_{eff} ratio increased from 0.14 to 0.71 after low-temperature hydrogenation and rose further to 1.20 upon high-temperature hydrogenation. Low-temperature hydrogenation was carried out at 398 K because it was found to be the lowest temperature at which all the pyrolysis oil functionalities started showing substantial activity toward hydrogenation. The compositions of the low-temperature hydrogenated WSBO and high-temperature hydrogenated WSBO, based on chromatographic analysis, are shown in Fig. 3 and in more detail in table S4. In the low-temperature hydrogenation, we were able to convert 29.4% of the WSBO feed car-

bon to gasoline cut 1, gasoline cut 2, and C2 to C6 diols (Fig. 3B), which can be marketed as products in their own right. Gasoline cut 1 comprises mainly small (up to three carbon atoms) monohydric alcohols that boil in the temperature range of 338 to 373 K. Gasoline cut 2 mainly contains C4 to C6 monohydric alcohols that boil in the temperature range of 388 to 448 K. The C2 to C6 diols boil above 450 K, with ethylene glycol and propylene glycol being the major components. These product groups can easily be separated by distillation. Gasoline cut 1 and gasoline cut 2 can be added to gasoline as renewable high-octane additives. Glycols can be further purified and sold. The thermal stability of the WSBO increases substantially after hydrogenation, with a homogeneous thermal coke yield decreasing from 12.8 wt % for the WSBO to 2.8 wt % for the WSBO after a low-temperature hydrogenation. The hydrogen consumption for the low-temperature hydrogenation process was 4.8 g of H_2 /100 g of carbon in the feed.

However, the product distribution still contains substantial amounts of hydrogen-deficient compounds such as acetic acid (H/C_{eff} ratio = 0), levoglucosan (H/C_{eff} ratio = 0), and sorbitol

Table 1. Overall carbon yield for the integrated catalytic process for the conversion of pyrolysis oils and the aqueous fraction of pyrolysis oil. Carbon selectivity values are reported for the individual components benzene, toluene, xylene, ethylbenzene (EtBenz), ethylene, propylene, and butylene.

Feed	Process	Hydrogen consumption (g/100 g of carbon in feed)	Final products (% carbon yield) or carbon selectivity (%)					
			C1 to C6 alkanes	CO _x	Aromatics	Olefins	Coke	Unidentified
DOE-BO	Zeolite	None	0	18.3	9.8	11.2	49.5	11.2
					Carbon selectivity (%)			
					Benzene: 17.3	Ethylene: 51.8		
					Toluene: 40.8	Propylene: 36.6		
					Xylene: 23.5	Butylene: 11.6		
					EtBenz: 2.0			
DOE-BO	Ru/H ₂ + Zeolite	0.9	2	26.5	14.4	18.2	34.6	4.3
					Carbon selectivity (%)			
					Benzene: 16.9	Ethylene: 52.2		
					Toluene: 37.2	Propylene: 35.9		
					Xylene: 38.5	Butylene: 11.4		
					EtBenz: 3.4			
WSBO	Zeolite	None	0	23.7	8.2	18.5	32.3	17.3
					Carbon selectivity (%)			
					Benzene: 26.8	Ethylene: 41.6		
					Toluene: 46.3	Propylene: 45.9		
					Xylene: 20.7	Butylene: 12.4		
					EtBenz: 1.2			
WSBO	Ru/H ₂ + Zeolite	4.8	7.5	13.9	21.6	30.2	17.4	9.4
					Carbon selectivity (%)			
					Benzene: 17.6	Ethylene: 31.8		
					Toluene: 45.5	Propylene: 55.4		
					Xylene: 31.3	Butylene: 12.8		
					EtBenz: 2.6			
WSBO	Ru/H ₂ + Pt/H ₂ + Zeolite	8.1	15.0	10.7	18.3	43.0	12.6	0.4
					Carbon selectivity (%)			
					Benzene: 27.0	Ethylene: 32.0		
					Toluene: 49.3	Propylene: 53.8		
					Xylene: 19.1	Butylene: 14.2		
					EtBenz: 2.3			

(H/C_{eff} ratio = 0.33). By feeding this stream through a high-temperature hydrogenation reactor at 523 K and 100 bar, the total carbon yield of gasoline cut 1, gasoline cut 2, and C2 to C6 diols increased to 45.8% (Fig. 3C and table S4). In this process, the product distribution can be customized by modifying the reaction conditions; for example, if there is a larger market for gasoline cut 1, its yield can be increased by operating the second stage at higher temperatures or lower space velocities. At higher temperatures, C2 to C6 diols undergo further C-O and C-C bond cleavage reactions, producing small monohydric alcohols. High-temperature hydrogenation was carried out at 23 K so as to achieve high sorbitol and levoglucosan conversion. In the high-temperature hydrogenation step, a 100% levoglucosan conversion and a 95% sorbitol conversion were obtained. An increase in carbon yield to the desired product groups of gasoline cut 1, gasoline cut 2, and C2 to C6 diols was observed, corresponding to the 80% of the levoglucosan and sorbitol carbon. Sorbitol can be converted over a supported Pt catalyst to a mixture of alcohols and polyols through reactions including hydrogenation, dehydration, decarbonylation, and retro-aldol condensation (32). Although sorbitol is substantially more thermally stable than glucose, it still has a low overall H/C_{eff} ratio of 0.33 in comparison to the H/C_{eff} ratios of the possible products from hydrogenolysis of sorbitol, including propylene glycol (H/C_{eff} ratio = 1.33), propanol (H/C_{eff} ratio = 2), and butanediols (H/C_{eff} ratio = 1.5). The net hydrogen consumption for the combined low- and high-temperature hydrogenation process was 8.1 g of H_2 /100 g of carbon in the feed. The WSBO had high thermal stability after the high-temperature hydrogenation, with the homogeneous thermal coke yield decreasing from 12.8 wt % for the WSBO to 0.2 wt % after high-temperature hydrogenation. High-pressure hydrogen is necessary in the high-temperature

step to minimize carbon loss to less valuable C1 to C4 alkanes (32, 34).

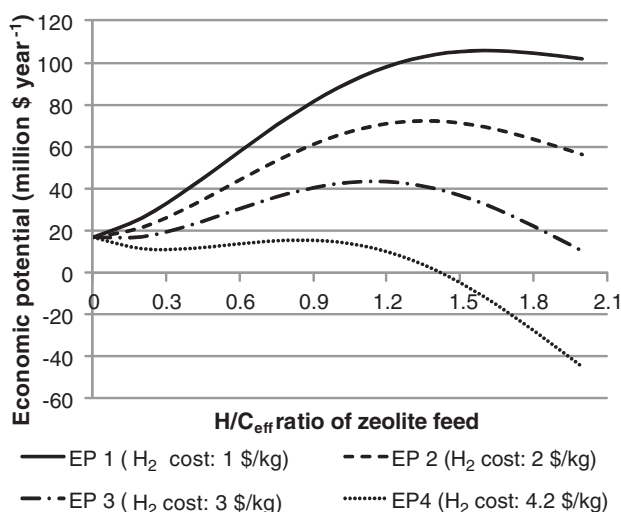
These hydrogenated feeds were then added to the zeolite step (step yields are in Fig. 3D and overall yields in Table 1). Only 20% of the DOE-BO carbon was converted to olefins and aromatics if fed directly to the zeolite without any hydrotreatment, with 50% of the carbon forming coke. The remaining 30% of the carbon was converted to CO, CO_2 , and unidentified oxygenates. Low-temperature hydrogenation raises the yield of olefins and aromatics after zeolite upgrading to 32.6% (Table 1). The low-temperature hydrogenation step only used a small amount of hydrogen but resulted in a substantial increase in the yield of desired products. Similar results were observed with the water-soluble fraction of the WSBO. Direct zeolite upgrading of WSBO affords 26.7% carbon yield of olefins and aromatics. Low-temperature hydrogenation before zeolite upgrading raised the yield to 51.8%, whereas the high-temperature hydrogenation leads to 61.3% olefins and aromatics yield. The high-temperature hydrogenation process converts 15.0% of the carbon in WSBO to light alkanes, which is twice that of the low-temperature hydrogenation process. All these points are shown in Fig. 1 along with the theoretical yield, with toluene as the major product. The difference between experimental and theoretical yields is reduced with the increasing H/C_{eff} ratio of the WSBO feed, indicating that the hydrogen added is used primarily for increasing the olefin and aromatics yield from the process. As seen in table S8, the percentage of theoretical toluene yield for the water-soluble fraction of the WSBO increased from 39.1 to 64.9% upon low-temperature hydrogenation and increased further to 72.3% upon high-temperature hydrogenation.

The major products obtained in the zeolite upgrading of DOE-BO and WSBO are C2 to C4 olefins and C6 to C8 aromatic hydrocarbons.

Under the reaction conditions used in this study, the olefin selectivity for the DOE-BO and low-temperature hydrogenated DOE-BO was 52% to ethylene and 36% to propylene, with the balance being butylenes. The aromatic selectivity decreased with toluene (37 to 40% selectivity) > xylenes (23.5 to 38.5% selectivity) > benzene (17% selectivity). The olefin selectivity for the WSBO and hydrogenated WSBO was different than that of the DOE-BO, with an olefin selectivity of 45 to 55% to propylene and 32 to 42% to ethylene, with the balance being butylenes. The aromatic selectivity decreased with toluene (45 to 50% selectivity) > xylenes (20 to 30% selectivity) > benzene (17 to 27% selectivity). As we have demonstrated previously, the ratios of these products can be tuned by adjusting both the reaction conditions and catalytic process (35). For example, in the zeolite upgrading of high-temperature hydrogenated WSBO, the aromatic hydrocarbon-to-olefin ratio increases from 1:2.8 to 1:1.1 as the temperature is reduced from 923 to 673 K (table S6). Olefins can also be converted to aromatics by recycling the olefins back to the zeolite reactor. Several existing processes can also be used to convert olefins to aromatics, including olefin aromatization and alkylation of the aromatics using olefins (36, 37). These results indicate that the olefin-to-aromatic ratio and the types of olefins and aromatics produced can be adjusted according to the market demand, using several approaches.

The cost of hydrogen is important in determining how much hydroprocessing should be done before deoxygenation with the zeolite catalyst. Figure 4 depicts the economic potential of our integrated catalytic process as a function of the H/C_{eff} ratio of the feed-to-zeolite step for four different hydrogen costs. The economic potential of the process is calculated by subtracting the cost of process feeds (biomass and hydrogen) from the selling price of the products. Importantly, the economic potential includes only the costs of the raw material and does not include any other operating costs or capital costs. The market price of hydrogen varies widely depending on location, mode of supply, and natural gas prices. The cheapest hydrogen is from large steam reformers and typically costs \$1.50 to \$2.50/kg (38), whereas the hydrogen shipped in tube trailers can cost as much as \$12/kg (39). The optimum H/C_{eff} ratio, where the economic potential of the process is highest, decreases with increasing hydrogen cost. For example, if hydrogen cost is \$2/kg, then the maximum economic potential occurs at an H/C_{eff} of 1.4, whereas when the hydrogen cost is \$4.20/kg, the maximum economic potential occurs when pyrolysis oil is fed directly to the zeolite catalyst without any hydrogenation. Combining the hydrogenation steps with a zeolite conversion step reduces the overall hydrogen requirements as compared to using hydrogen for a complete deoxygenation of pyrolysis oil. A complete deoxygenation of pyrolysis oil by hydrodeoxygenation, with a 100% carbon yield to the corresponding hydrocarbons

Fig. 4. Annual economic potential (EP) for the integrated hydroprocessing and zeolite upgrading of pyrolysis oil as a function of the H/C_{eff} ratio of feed to zeolite catalyst. Data in Fig. 1 were used for calculating the economic potential values. The plant capacity was assumed to be 100 metric tons/hour of biomass. Pyrolysis oil yield was assumed to be 70 wt % of biomass. It was assumed that in the zeolite upgrading step, all olefins produced are converted to aromatics. A July 2010 spot price of benzene of \$2.60/gallon was used as the aromatic hydrocarbon selling price (43). Biomass cost was assumed to be \$50/ton.



(that is, alkanes from C1 to C6 nonphenolic oxygenates and aromatics from phenolic compounds), requires 14 to 15 g of H₂/100 g of carbon in the feed, if the catalyst coking problems are overcome. In comparison, increasing the H/C_{eff} ratio of pyrolysis oil from 0 to 1.4 requires 11.7 g of H₂/100 g of carbon in the feed, reducing the hydrogen requirements as compared to complete hydrodeoxygenation by 20%. Furthermore, during the hydrotreating process, large amounts of undesired methane are produced, which also can substantially increase the hydrogen requirements. Hydrogen required in these processes should preferably be obtained from renewable sources, such as by the reforming of biomass-derived feedstock (14, 40, 41). Alternatively, hydrogen can be obtained from coal gasification or from water splitting driven by carbon-free energy sources, such as solar, nuclear, and wind energy, as suggested by Agrawal *et al.* (42). Zeolite catalysts convert the biomass feedstocks into aromatics and olefins, which can fit easily into the existing infrastructure. Increasing the yield of petrochemical products from biomass therefore requires hydrogen. Thus, there exists an optimum solution for the economical maximum yield of petrochemical feedstocks products that is dictated by the cost of hydrogen. It is expected that future advances in the field of metal and zeolite catalysts, combined with reaction engineering, will allow us to design even more efficient and economical processes to convert biomass resources to renewable chemical industry feedstocks.

References and Notes

- A. J. Ragauskas *et al.*, *Science* **311**, 484 (2006).
- G. W. Huber, S. Iborra, A. Corma, *Chem. Rev.* **106**, 4044 (2006).
- E. L. Kunkes *et al.*, *Science* **322**, 417 (2008).
- D. L. Klass, *Biomass for Renewable Energy, Fuels and Chemicals* (Academic Press, San Diego, CA, 1998).
- S. Czernik, A. V. Bridgwater, *Energy Fuels* **18**, 590 (2004).
- D. Mohan, C. U. Pittman, P. H. Steele, *Energy Fuels* **20**, 848 (2006).
- M. M. Wright, D. E. Dugaard, J. A. Satrio, R. C. Brown, *Fuel* 10.1016/j.fuel.2010.07.029.
- R. P. Anex *et al.*, *Div. Fuel Chem.* **54**, 704 (2009).
- A. Oasmaa, S. Czernik, *Energy Fuels* **13**, 914 (1999).
- D. C. Elliott, *Energy Fuels* **21**, 1792 (2007).
- T. L. Marker *et al.*, *Opportunities for Biorenewables in Petroleum Refineries* (Final Report, DE-FG36-05G015085, UOP, Des Plaines, IL, 2005); www.osti.gov/bridge/servlets/purl/861458-Wv5uum/861458.pdf.
- J. D. Adjaye, N. N. Bakhshi, *Fuel Proc. Tech.* **45**, 161 (1995).
- J. D. Adjaye, N. N. Bakhshi, *Fuel Proc. Tech.* **45**, 185 (1995).
- T. P. Vispute, G. W. Huber, *Green Chem.* **11**, 1433 (2009).
- C. H. Bartholomew, R. J. Farrauto, *Fundamentals of Industrial Catalytic Processes* (Wiley, Hoboken, NJ, 2006).
- T. F. Degnan Jr., M. Smith, C. R. Venkat, *Appl. Catal. A* **221**, 283 (2001).
- C. Perego, P. Ingallina, *Catal. Today* **73**, 3 (2002).
- G. R. Lappin, J. D. Sauer, Eds., *Alpha Olefins Applications Handbook* (Marcel Dekker, New York, NY, 1989).
- H. H. Zsmant, *Organic Building Blocks of the Chemical Industry* (Wiley, New York, 1989).
- N. Y. Chen, T. F. Degnan Jr., L. R. Koenig, *Chemtech* **16**, 506 (1986).
- A. G. Gayubo, A. T. Aguayo, A. Atutxa, R. Aguado, J. Biolbao, *Ind. Eng. Chem. Res.* **43**, 2610 (2004).
- T. R. Carlson, T. P. Vispute, G. W. Huber, *ChemSusChem* **1**, 397 (2008).
- R. Bayerbach, D. Meier, *J. Anal. Appl. Pyrolysis* **85**, 98 (2009).
- T. A. Milne, F. Agblevor, M. Davis, S. Deutch, D. Johnson, in *Developments in Thermal Biomass Conversion*, A. V. Bridgwater, D. G. B. Boocock, Eds. (Blackie Academic and Professional, London, 1997), pp. 409–424.
- J. S. Shabtai, W. W. Zmierzczak, E. Chornet, U.S. Patent 5,959,167 (1999).
- J. S. Shabtai, W. W. Zmierzczak, E. Chornet, U.S. Patent 6,172,272 (2001).
- J. E. Miller, L. Evans, A. Littlewolf, D. E. Trudell, *Fuel* **78**, 1363 (1999).
- C. Zhao, Y. Kou, A. A. Lemonidou, X. B. Li, J. A. Lercher, *Angew. Chem. Int. Ed.* **48**, 3987 (2009).
- S. Crossley, J. Faria, M. Shen, D. E. Resasco, *Science* **327**, 68 (2010).
- C. Zhao, Y. Kou, A. A. Lemonidou, X. B. Li, J. A. Lercher, *Chem. Commun.* **46**, 412 (2010).
- H. Olcay, L. Xu, Y. Xu, G. W. Huber, *ChemCatChem* 10.1002/cctc.201000134 (2010).
- N. Li, G. W. Huber, *J. Catal.* **270**, 48 (2010).
- Materials, methods, and calculations are available as supporting material on *Science* Online.
- G. W. Huber, R. D. Cortright, J. A. Dumesic, *Angew. Chem. Int. Ed.* **43**, 1549 (2004).
- T. R. Carlson, Y. Cheng, J. Jae, G. W. Huber, *Energy Environ. Sci.* 10.1039/C0EE00341G.
- M. Guisnet, N. S. Gnep, F. Alario, *Appl. Catal. A* **89**, 1 (1992).
- V. R. Choudhary, P. Devadas, S. Banerjee, A. K. Kinage, *Microporous Mesoporous Mater.* **47**, 253 (2001).
- C. E. G. Padro, V. Putsche, *Survey of the Economics of Hydrogen Technologies* (report, NREL/TP-570-27079, National Renewable Energy Laboratory, Golden, CO, 1999); <http://www1.eere.energy.gov/hydrogenandfuelcells/pdfs/27079.pdf>.
- National Research Council and National Academy of Engineering, *The Hydrogen Economy: Opportunities, Costs, Barriers, and R&D Needs* (National Academies Press, Washington, DC, 2004).
- R. D. Cortright, R. R. Davda, J. A. Dumesic, *Nature* **418**, 964 (2002).
- G. W. Huber, J. W. Shabaker, J. A. Dumesic, *Science* **300**, 2075 (2003).
- R. Agrawal, N. R. Singh, F. H. Ribeiro, W. N. Delgass, *Proc. Natl. Acad. Sci. U.S.A.* **104**, 4828 (2007).
- ICIS pricing report, www.icis.com/v2/chemicals/9075158/benzene/pricing.html.
- This work was supported by the U.S. Department of Energy Office of Energy Efficiency and Renewable Energy, under grant DE-FG36-08G018212; and by the Defense Advanced Research Projects Agency (DARPA) through the Defense Science Office Agreement HR0011-09-C-0075 (Approved for Public Release, Distribution Unlimited); the National Basic Research Program of China (973 Program, grant no. 2010CB732206); and the National Natural Science Foundation of China (grant no. 51076031). The views, opinions, and/or findings contained in this article/presentation are those of the author/presenter and should not be interpreted as representing the official views or policies, either expressed or implied, of the DARPA or the Department of Defense. We thank A. Javadi for the microfiltration of bio-oil, Y. Cheng for help with zeolite upgrading experiments, and the National Renewable Energy Laboratory and P. H. Steele from the Forest Products Department at Mississippi State University for providing us with bio-oil samples. The University of Massachusetts has filed a patent based on the methods presented here. G.W.H. has a financial interest in Anellotech, a privately held company focused on producing renewable petrochemicals from biomass.

Supporting Online Material

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Materials and Methods
Figs. S1 and S2
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The Core Structure of Basal Dislocations in Deformed Sapphire (α -Al₂O₃)

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The atomic structure of dislocation cores is decisive for the understanding of plasticity in crystalline solids. The core structure of dislocations in sapphire introduced by high-temperature plastic deformation has been investigated with the use of the negative spherical-aberration imaging technique. The ability of this technique to discriminate oxygen columns from aluminum (Al) columns, combined with reproduction of subtle contrast features by image simulation, leads to a markedly detailed atomic model of the dislocation cores. The partial dislocations are Al-terminated, with electrical neutrality being achieved because half of the Al columns are missing. These partials also undergo core spreading, which results in random occupancy of both tetrahedrally and octahedrally coordinated sites, though Al in tetrahedral coordination never occurs in a perfect crystal. Unusual dislocation core structures may be present in other technologically important nonmetallic solids.

Although ceramics are notorious for their brittle behavior under normal service conditions, plastic deformation in such materials can be realized at elevated temperatures, under circumstances in which friction and wear occur, and wherever cracking is suppressed by confining pressures, such as under an inden-

tation. Under all of these conditions, the behavior of dislocations, the principal “carrier” of plastic deformation in crystalline materials, assumes paramount importance.

Aluminum oxide (α -Al₂O₃) in both single-crystal form (sapphire) and as dense polycrystals finds utility in a wide range of important

applications, from laser hosts (Ti-sapphire) to inert ultrahard substrates. Plastic deformation at high temperatures by basal slip $\left((0001) \frac{1}{3} \langle 11\bar{2}0 \rangle \right)$ in sapphire ($\alpha\text{-Al}_2\text{O}_3$) was first reported by Wachtman and Maxwell in 1954 (1). Shortly thereafter, Kronberg (2) suggested that dissociation of basal dislocations into half partials could occur

$$\frac{1}{3} \langle 11\bar{2}0 \rangle \rightarrow \frac{1}{3} \langle 10\bar{1}0 \rangle + \frac{1}{3} \langle 01\bar{1}0 \rangle \quad (1)$$

with further dissociation into quarter partials also possible.

$$\frac{1}{3} \langle 10\bar{1}0 \rangle \rightarrow \frac{1}{9} \langle 2\bar{1}\bar{1}0 \rangle + \frac{1}{9} \langle 11\bar{2}0 \rangle \quad (2)$$

Equation 1 involves faulting only in the cation sublattice, as $\frac{1}{3} \langle 10\bar{1}0 \rangle$ is a translation vector of the anion sublattice. Nevertheless, the fault arising from reaction (1) on the basal plane has a very high

stacking fault energy (SFE), $\sim 1.5 \text{ J/m}^2$ (3), and such glide dissociation has never been observed. However, the $\frac{1}{3} \langle 10\bar{1}0 \rangle$ SFE is very anisotropic, and climb dissociation of basal dislocations at elevated temperatures is ubiquitous, due to the SFE being ~ 5 to 10 times smaller on the prism planes (4). Equation 2 involves an even higher SFE but may occur in the core of the $\frac{1}{3} \langle 01\bar{1}0 \rangle$ partials [core spreading (5)].

Figure 1A is a schematic representation of dislocation motion in sapphire. A 90° (edge) dislocation is shown gliding on a basal plane with a narrow stacking fault (width of $2b$, where b is the Burgers vector); this dislocation would appear undissociated if it could be imaged with a transmission electron microscope (6). The cores of the $\frac{1}{3} \langle 10\bar{1}0 \rangle$ partials have probably undergone core spreading, as suggested earlier (5), but again, this would not be discernable by conventional transmission electron microscopy (TEM). If a glissile dislocation were immobilized for any reason, the anisotropic SFE would lead to the configuration shown in Fig. 1A (iii), due to the much lower SFE for a $\frac{1}{3} \langle 10\bar{1}0 \rangle$ fault on $\{11\bar{2}0\}$ compared with (0001).

In proposing the importance of dislocation dissociation to the glide of basal dislocations in sapphire, Kronberg used an idealized version of

the structure of sapphire: a perfect hexagonal close-packed (hcp) oxygen sublattice and flat cation sheets. In reality, and as shown in supporting on-line material (SOM) fig. S1 (7), the oxygen sublattice is slightly distorted away from perfect hcp stacking, and the cation layers are “puckered.”

Although not discussed by Kronberg in his 1957 paper, this model of dislocation motion in sapphire required the motion plane to be between cation and anion layers and thus involves charge transport during dislocation motion. In spite of the fact that this must represent a substantial impediment to slip in a material with such strongly ionic bonding as $\alpha\text{-Al}_2\text{O}_3$, this aspect of dislocations in sapphire was ignored by Kronberg and the several other groups who subsequently studied deformation in $\alpha\text{-Al}_2\text{O}_3$ for the next four decades.

Using the actual, as opposed to the idealized, version of the crystal structure of sapphire, Bilde-Sørensen *et al.* (8) in 1996 presented a model for basal slip, again involving the $\frac{1}{3} \langle 10\bar{1}0 \rangle$ half partials of Eq. (1), but also a motion plane within the puckered cation layer. In this model, each $\frac{1}{3} \langle 10\bar{1}0 \rangle$ partial dislocation, as it moves to the half-slipped position, transports only half of the cations and, thus, does not involve any net charge transport, with the trailing partial restoring a

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A 90° Edge Dislocation

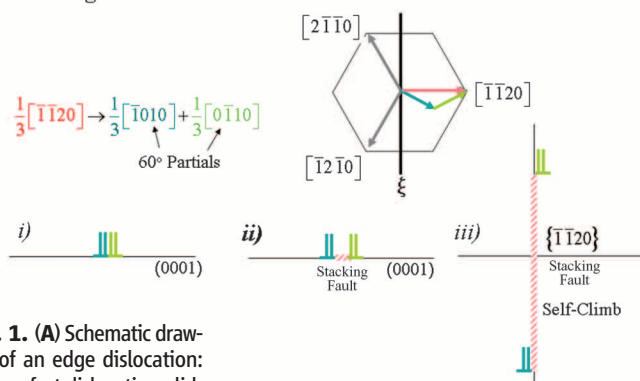
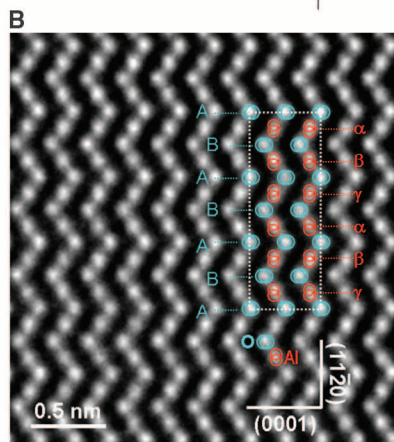
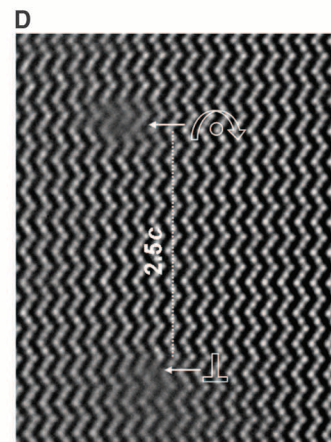
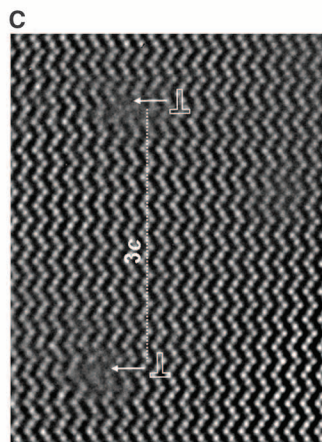
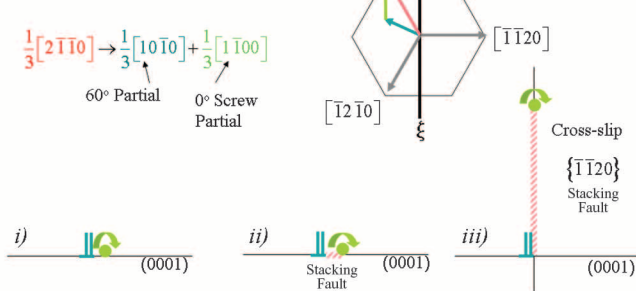


Fig. 1. (A) Schematic drawing of an edge dislocation: (i) a perfect dislocation gliding on the basal plane, (ii) hypothetical core spreading leading to a stacking fault of limited width, and (iii) after dissociation onto a $\{11\bar{2}0\}$ prism plane via self-climb. (B) NCSI image of sapphire along $[1\bar{1}00]$. The image shows a perfect crystal in a sample deformed to $\sim 5\%$ strain at 1400°C . The projection of the structure along $[1\bar{1}00]$ is superimposed on the image. Note that the oxygen columns in the image show brighter contrast than do the Al columns. (C) NCSI image of a climb-dissociated edge dislocation in the foil used for (B). Arrows indicate the cores of the partial dislocations. (D) NCSI image of a 30° dislocation. (E) Schematic drawing of a 30° mixed dislocation: (i) a perfect dislocation



E 30° Dislocation



gliding on the basal plane, (ii) hypothetical core spreading leading to a stacking fault of limited width, and (iii) after dissociation onto a $\{11\bar{2}0\}$ prism plane via cross-slip.

perfect lattice. Even though the SFE for basal slip is too large to allow resolvable glide dissociation (6), the Bilde-Sørensen *et al.* model describes the atomic motion within the dislocation core as the dislocation moves one Burgers vector.

Shibata *et al.* (9) used scanning TEM to study climb-dissociated basal dislocations within a small-angle bicrystal boundary in sapphire and concluded that the core structures of the dissociated dislocations were nonstoichiometric and therefore electrically charged. They further suggested that these charged, nonstoichiometric partials were characteristic of glissile basal dislocations that are active during high-temperature plastic deformation in α -Al₂O₃, and they developed a variant of Kronberg's synchroshear model to describe the motion of the nonstoichiometric partials. They did not consider the implications of charged dislocations on basal slip in sapphire.

Lagerlöf *et al.* (5) subsequently considered the implications of nonstoichiometric and charged dislocation cores on the motion of glide dislocations in a bulk sapphire crystal undergoing basal deformation at elevated temperatures and concluded that such nonstoichiometric dislocations cores are unlikely to be involved in high-temperature basal deformation. However, it may be possible for nonstoichiometric charged dislocation cores to exist in a periodic array in a manufactured small-angle boundary.

The purpose of the present work was to determine the core structure of basal dislocations in a sapphire single crystal deformed to ~5% strain at 1400°C with the use of the negative spherical-aberration imaging (NCSI) technique (10). In the image of a perfect crystal from such a specimen

shown in Fig. 1B, the projection of the sapphire structure along the $[1\bar{1}00]$ viewing direction is superimposed on the image, allowing identification of the atomic positions of both oxygen and Al columns. Along this viewing direction, the puckered Al cation layers are seen to consist of pairs of columns in the structure projection. In the image, the pairs of Al columns in the puckered layers cannot be resolved due to the resolution limit of the instrument (~80 pm).

An NCSI image of an edge dislocation that had dissociated by climb into two $\frac{1}{3}\langle 10\bar{1}0 \rangle$ partials is shown in Fig. 1C; these must be 60° partials. We have previously measured the SFE of climb-dissociated edge dislocations with conventional TEM (11) and found (from the separation of $\frac{1}{3}\langle 10\bar{1}0 \rangle$ partials) the SFE to be between 0.1 and 0.25 J/m²; the image in Fig. 1C (assuming the partials are at a friction-free equilibrium) suggests a SFE of 0.21 J/m² (12).

Other dislocations in this TEM foil appeared with a single $\frac{1}{3}\langle 10\bar{1}0 \rangle$ partial visible (Fig. 1D). The second partial must have either a Burgers vector parallel to the viewing direction ($\frac{1}{3}\langle 10\bar{1}0 \rangle$) or pure screw character, as such an image would not be possible for a pure edge dislocation. Given the edge component visible at the bottom of the image, this dislocation must be a 60° $\frac{1}{3}\langle 10\bar{1}0 \rangle$ partial. At a distance of 2.5c (where c is the unit cell parameter along $\langle 0001 \rangle$) above the $\frac{1}{3}\langle 10\bar{1}0 \rangle$ partial, faint contrast is evident, similar to that of the dislocation core in Fig. 1C. However, there is no edge component of the Burgers vector at this position. We believe that the dislocation imaged in Fig. 1D is a 30° dislocation that had dissociated to a 60° partial and a screw partial; the config-

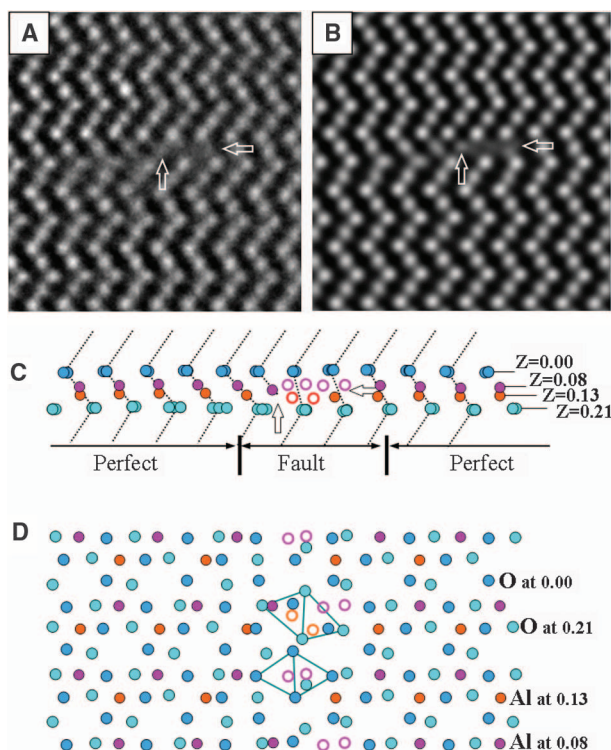
uration imaged in this figure probably formed by cross slip after the 30° glissile dislocation had been immobilized (Fig. 1E). The separation of the two partials suggests a SFE of 0.17 J/m² (13).

We focused on these 60° $\frac{1}{3}\langle 10\bar{1}0 \rangle$ partials for determining the dislocation core structure at atomic resolution; in particular, the images of such 60° partials contained a similar set of contrast features (see fig. S2). These include (i) modest contrast of the terminating Al columns in comparison with the equivalent columns on the left-hand side of the dislocations; (ii) strongly reduced contrast of the Al columns on the right-hand side of the terminating columns, in comparison with the equivalent columns in areas of perfect lattice, as denoted by the lateral arrows; and (iii) similar image contrast of the oxygen columns at the cores compared with that in perfect crystal.

The common image-contrast features at the dislocation cores have been successfully reproduced by means of quantum-mechanical and optical image simulation (14). As a representative example, the experimental image of Fig. 2A has been selected for direct comparison with image simulation. A trial defect structure is assumed, and images are calculated by solving the Schrödinger equation for the electron wave function, taking into account effects of specimen thickness and crystal tilt. The image intensity distribution is calculated, also considering the effects of residual lens aberrations. By the use of an established comparison procedure (15), the atomic structure model is modified in an iterative manner until an optimum fit to the experimental image-contrast distribution is obtained (Fig. 2B). Furthermore, surface relaxation around a dislocation intersecting a free surface, the so-called Eshelby twist (16–19), is possible in a TEM foil because of the shear stresses associated with the screw component of the dislocation. This effect has been included in the simulation.

The atomic positions at the dislocation core are displayed in a $[1\bar{1}00]$ projection in Fig. 2C and in a basal plane projection in Fig. 2D; the core extends over four atomic planes. For clarity, three (0001) atomic layers, including the dislocation core and involving the puckered Al layer sandwiched between two oxygen layers, are shown in Fig. 2C. The displacements due to the extra half plane of the partial dislocation are confined to the two lattice planes on either side of the partial dislocation; a number of Al atomic columns—those shown as open circles—have 50% occupancy of the Al cations with respect to a perfect lattice. Some of the Al cations are in tetrahedral coordination in the dislocation core, and the Al cations are randomly distributed between sites with tetrahedral and octahedral coordination. The model of the core structure clearly shows that the low contrast of the terminating Al column at the dislocation core is caused by the absence of half of a column pair. The reduced contrast of the Al columns on the right-hand side of the terminating Al column arises from relative displacement of the Al atoms away from the regular column positions.

Fig. 2. Experimental (A) and simulated (B) images of a 60° partial dislocation in deformed sapphire. The simulated image is calculated for a sample thickness of 4.1 nm, defocus = 5 nm, two-fold astigmatism = 2 nm, three-fold astigmatism = 30 nm, coma = 30 nm, crystal tilt of 2.0 mrad, and vibration of 0.03 nm. The simulation is based on a structure model with Eshelby twist. The details of the dislocation core are shown in the $[1\bar{1}00]$ projection in (C) and in a basal projection in (D). Open circles denote 50% occupancy. Vertical arrows indicate the modest contrast of the terminating Al column; horizontal arrows indicate the strongly reduced contrast of the Al columns on the right-hand side of the terminating column. Z denotes relative positions in nanometers.



On the basis of the satisfactory fit of the structure model to the experimental image, we suggest that the dislocation cores are neither charged nor nonstoichiometric. We note, however, that small changes in contrast occur at the individual dislocation cores in these 60° partials, which must reflect the randomness of the Al atoms in tetrahedral and octahedral coordination near the dislocation core (see fig. S2). In turn, these changes must depend on details of the associated local strain and lattice distortion.

The notion of Al^{3+} in tetrahedral coordination at a dislocation core was first suggested by Lagerlöf *et al.* (5) in a discussion of core spreading of a partial dislocation. They assumed that Eq. 2 occurred in the core of the $\frac{1}{3}\langle 10\bar{1}0 \rangle$ partials

and argued that steric constraints were sufficiently modest that such core spreading was likely. Figure 2 provides good evidence that their speculation was correct.

Assuming that the core structure portrayed in Fig. 2 is correct, we can now provide plausible atomistic models for glissile edge and 30° dislocations in sapphire. Consider an edge dislocation formed by the removal of two planes of atoms (as shown in Fig. 3, A and B) viewed along $[1\bar{1}00]$ and $[000\bar{1}]$, respectively, or the similarly formed 30° dislocation (shown in Fig. 3, C and D) (20). (Perfect crystal in these orientations is shown in fig. S1, B and C.) The terminating plane of Al^{3+} ions shows only 50% occupancy, and the spreading of the cores of the $\frac{1}{3}\langle 10\bar{1}0 \rangle$ partials results in

tetrahedral coordination of the Al^{3+} cations (compare with Fig. 2).

The structure models in Fig. 3 make clear that the relative displacements present in the dislocated crystal occur in the puckered Al layer (that is, between the two halves of the column pairs), consistent with the Bilde-Sørensen *et al.* model (8). The puckered Al layer consists of upper and lower sublayers of Al cations parallel to (0001) . The upper half layer is strongly bonded to the oxygen layer above it and the lower half layer to the oxygen layer below it. Because of the shear stress introduced by the dislocation, insertion or removal of half of an atomic plane causes a relative displacement between the two parts above and below the center of the dis-

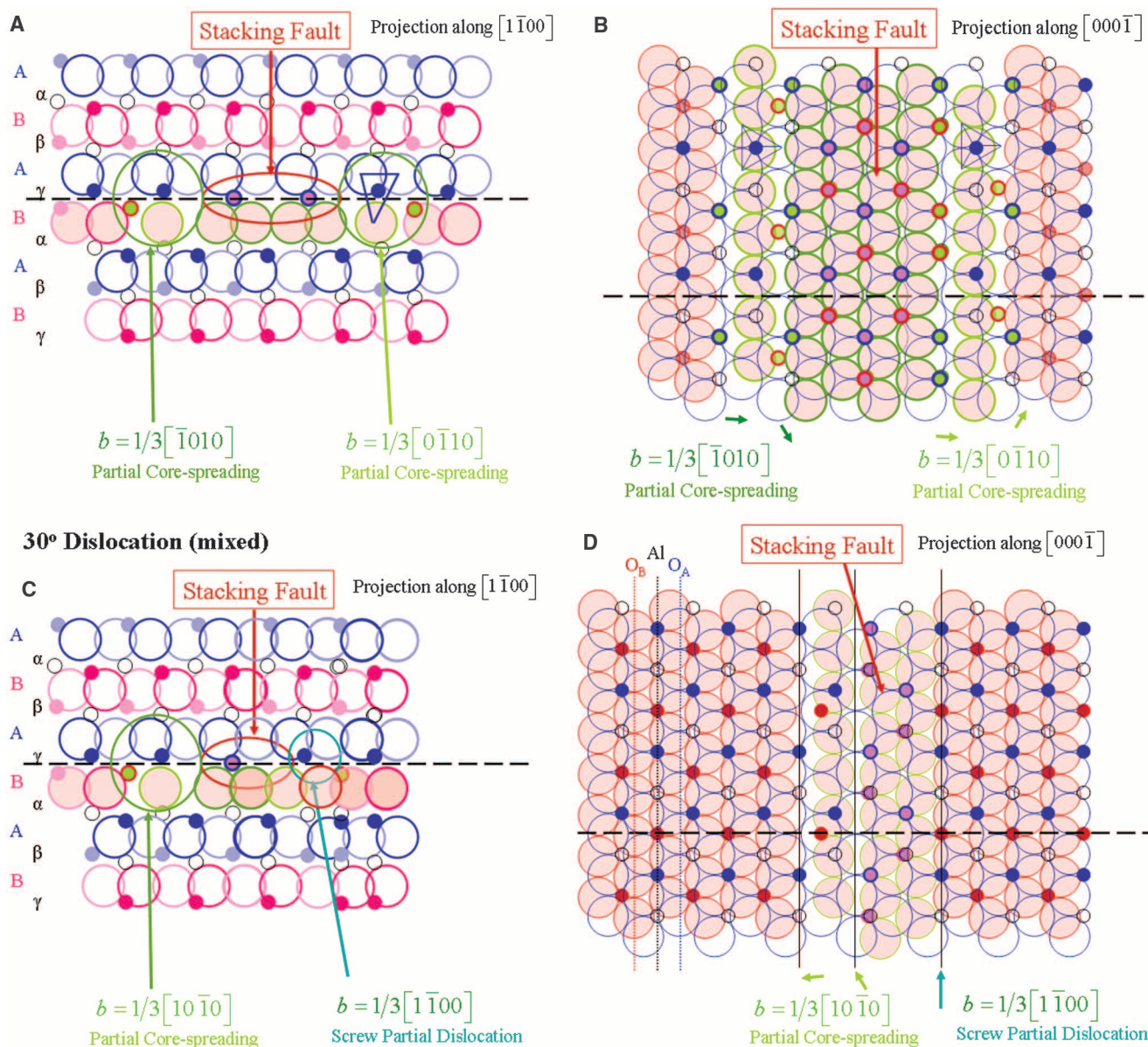


Fig. 3. Schematic representations of the structure of Al_2O_3 viewed along $[1\bar{1}00]$ (A) and (C) and also along $[000\bar{1}]$ (B) and (D), after removal of two planes of atoms. (A) and (B) show an edge partial, whereas (C) and (D) show a 30° partial. Note that a stacking fault of width $2b$ is assumed,

as is spreading of the cores of the $\frac{1}{3}\langle 10\bar{1}0 \rangle$ partials. The small open circles are normally empty octahedral interstitial sites. The triangles in (A) and (B) denote Al in tetrahedral coordination. O_A and O_B denote oxygen atoms in alternating layers.

location core, which occurs in the plane with relatively weak bonding (that is, between the upper and lower layers of the puckered Al layer). As no charge transport is involved, the Peierls barriers are smaller than if the dislocations were charged.

It is not clear whether the core structure of glissile basal dislocations shown in Fig. 3 should be expected for dislocations present in a low-angle boundary (9). The periodic electrostatic and stress fields present in such a boundary could well lead to a different core structure for its constituent dislocations.

The NCSI studies have also revealed a stacking fault on the basal plane (fig. S4), but only in the thinnest regions of the TEM foils; such faults have not been reported for α -Al₂O₃ as yet. Partial dislocations are present at the ends of the three distinct faults in this image. At the dislocation cores, similar contrast to that of the dislocation core in Fig. 2A is observed: Low contrast occurs in the puckered Al layer. The details of the contrast feature of the experimental image have been reproduced in the calculated image, indicating a rearrangement of the Al atoms in the puckered layer (fig. S5) (7).

The NCSI technique has been used to image, at atomic resolution, partial dislocations bounding prism-plane stacking faults in sapphire deformed at high temperatures. The dislocations appear to be stoichiometric and uncharged, and the cores exhibit 50% occupancy of the Al cations to achieve electrical neutrality. Because of core spreading, the Al ions at the termination of the extra half planes exhibit random occupancy of

sites showing tetrahedral and octahedral coordination. It is likely that the motion plane is within the puckered Al sublattice, following the Bilde-Sørensen *et al.* model (8) for gliding basal dislocations.

References and Notes

1. J. B. Wachtman Jr., I. H. Maxwell, *J. Am. Ceram. Soc.* **37**, 291 (1954).
2. M. L. Kronberg, *Acta Metall.* **5**, 507 (1957).
3. A. G. Marinopoulos, C. Elsässer, *Philos. Mag. Lett.* **81**, 329 (2001).
4. M. H. Jhon, A. M. Glaeser, D. C. Chrzan, *Phys. Rev. B* **71**, 214101 (2005).
5. K. P. D. Lagerlöf, J. Castaing, A. H. Heuer, *Philos. Mag.* **89**, 489 (2009).
6. Actually, the conventional concept of a well-defined stacking fault with a well-defined SFE may break down when the energy is as high as it is for sapphire on the basal plane. The configuration in Fig. 1A (ii) should be thought of as an extended core.
7. Supporting material is available on Science Online.
8. J. B. Bilde-Sørensen *et al.*, *Acta Mater.* **44**, 2145 (1996).
9. N. Shibata *et al.*, *Science* **316**, 82 (2007).
10. C. L. Jia, M. Lentzen, K. Urban, *Science* **299**, 870 (2003).
11. K. P. D. Lagerlöf *et al.*, *Acta Metall.* **32**, 97 (1984).
12. Theoretical density functional theory calculations (4) yielded (0 K) values of 0.42 or 0.35 J/m², depending on the computational scheme that was employed (local density approximation or generalized gradient approximation). This difference from the experimental value might be due to the neglect of temperature effects in the calculations. Shell-model calculations (4) of SFEs indicate reductions of 0.1 to 0.2 J/m² between 0 and 1800 K due to vibrational entropy effects.
13. The small difference between the SFE calculated from the separation of the dissociated edge and 30° partials is possibly due to the partials not having reached their equilibrium separation.
14. D. B. Williams, C. B. Carter, *Transmission Electron Microscopy* (Springer, Berlin, ed. 2, 2009).
15. C. L. Jia *et al.*, *Nat. Mater.* **7**, 57 (2008).
16. J. D. Eshelby, A. N. Stroh, *Philos. Mag.* **42**, 1401 (1951).
17. As discussed in the SOM (fig. S3), the simulations of the dislocation core structure with and without consideration of the Eshelby twist (fig. S3, B and C) are indistinguishable. This arises because of the very small displacements involved in the Eshelby twist (which displacements are not included in Fig. 2, C and D, but were included in Fig. 2B to allow comparison with the experimental image).
18. Although α -Al₂O₃ has a trigonal crystal structure (space group no. 167: $R\bar{3}c$, where R is the rhombohedral space lattice), it is remarkably isotropic elastically [the anisotropy factor is approximately $A = 0.857$ [see, for example, (19)]]. Hence, the Eshelby-Stroh formula, which applies to elastic isotropic solids, can be used to a first approximation.
19. D. S. Phillips, T. E. Mitchell, A. H. Heuer, *Philos. Mag. A* **45**, 1371 (1982).
20. Note that the dislocations are again portrayed to have dissociated to a width of $\sim 2b$, and the cores of both partials have undergone core spreading.
21. We are grateful to L. Houben (ER-C, Jülich, Germany) for his help in modeling the Eshelby twist for the 60° partial dislocation and to K. Urban (ER-C, Jülich, Germany), M. Rühle (Max Planck Institute for Metals Research, Stuttgart, Germany), F. Ernst [Case Western Reserve Univ. (CWRU), Cleveland, OH], and P. Pirouz (CWRU, Cleveland, OH) for useful discussions.

Supporting Online Material

www.sciencemag.org/cgi/content/full/330/6008/1227/DC1

SOM Text

Figs. S1 to S5

References

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How Cats Lap: Water Uptake by *Felis catus*

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Animals have developed a range of drinking strategies depending on physiological and environmental constraints. Vertebrates with incomplete cheeks use their tongue to drink; the most common example is the lapping of cats and dogs. We show that the domestic cat (*Felis catus*) laps by a subtle mechanism based on water adhesion to the dorsal side of the tongue. A combined experimental and theoretical analysis reveals that *Felis catus* exploits fluid inertia to defeat gravity and pull liquid into the mouth. This competition between inertia and gravity sets the lapping frequency and yields a prediction for the dependence of frequency on animal mass. Measurements of lapping frequency across the family Felidae support this prediction, which suggests that the lapping mechanism is conserved among felines.

Terrestrial animals have evolved diverse means to acquire water, including absorption through the skin (1) or extraction of moisture from food (2), but most rely on drinking (3–12). Drinking presents a challenge to land vertebrates, because fresh water occurs mainly as horizontal liquid surfaces, such as puddles, ponds, lakes, or streams, and animals must displace water upward against gravity to drink it. Crucial in the drinking process is the role of the tongue, which in vertebrates is used in two distinctly different ways. Vertebrates with complete cheeks, such as pigs,

sheep, and horses, use suction to draw liquid upward and use their tongue to transport it intraorally (13, 14). In contrast, vertebrates with incomplete cheeks, including most carnivores, are unable (after weaning) to seal their mouth cavity to generate suction and must rely on their tongue to move water into the mouth (13). When the tongue sweeps the bottom of a shallow puddle, the process is called licking (4). When the puddle is deeper than the tongue excursion into the liquid, it is called lapping (15). Here, we report on the lapping mechanism of the domestic cat (*Felis catus*).

Almost everyone has observed a domestic cat lap milk or water. Yet casual observation hardly captures the elegance and complexity of this act, as the tongue's motion is too fast to be resolved by the naked eye. We used high-speed imaging to capture the motion of both the tongue and liquid during lapping [Fig. 1 and movie S1 (16)]. With the cat's face oriented downward, the tongue extends from the jaw (Fig. 1A) and its tip curls sharply caudally (Fig. 1B). At the lowest position of the tongue's tip, its dorsal side rests on the liquid surface, without piercing it (Fig. 1B). When the cat lifts the tongue, liquid adhering to the dorsal side of the tip is drawn upward, forming a column (Fig. 1C). This liquid column is further extended by the tongue's upward motion (Fig. 1D), thinning in the process (Fig. 1E), and is finally partially captured upon jaw closure (Fig. 1, E and F). Inside the mouth, cavities

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between the palate's rugae and the tongue act as a nonreturn device and trap liquid until it is ingested every 3 to 17 cycles (15).

This sequence of events reveals two important aspects of how *F. catus* laps. First, lapping does not rely on "scooping" water into the mouth as in dogs (*Canis lupus familiaris*). Although the dog's tongue also curls caudally in a cuplike shape, it penetrates the liquid surface and scoops up the water that fills its ventral side. In contrast, the cat's tongue does not dip into the liquid (Fig. 1B) and the cavity on its ventral side remains empty (Fig. 1, B and C), as also recognized in a 1940 Oscar-winning short film (17). Second, only the tip of the tongue is used for lapping. The tip is free of filiform papillae (18) (Fig. 1G), the semirigid hairlike structures that give a cat's tongue its characteristic roughness. Thus, papillae appear to have no function in lapping.

The movies allowed us to quantify the lapping kinematics. The position of the tip of the tongue was tracked over one lapping event (Fig. 2A) and averaged over 11 cycles (Fig. 2B). The tongue's vertical position during upward motion is well described by an error-function profile, $Z(t) = \frac{1}{2}H\{1 + \text{erf}(U_{\text{MAX}}t/\sqrt{\pi}H)\}$, where $H = 3.0$ cm (Fig. 2B, red) is the total vertical displacement, U_{MAX} is the maximum speed, and t is the time measured with respect to $Z(t=0) = H/2$, the half-height. As a result, the vertical velocity has an approximately Gaussian profile (Fig. 2B, blue): The tongue accelerates as it leaves the water surface, attains a remarkable maximum speed of $U_{\text{MAX}} = 78 \pm 2$ cm s⁻¹, then decelerates as it enters the mouth. Recordings of 10 adult individuals (16) yielded a lapping frequency $f = 3.5 \pm 0.4$ s⁻¹ and an ingested volume per lap $V = 0.14 \pm 0.04$ ml.

To help understand the mechanism of lapping, we performed physical experiments in which a glass disk of radius R (representing the tongue's tip), initially placed on a water surface, was pulled vertically upward (Fig. 3). The time series of the disk position was set by a computer-controlled

stage and programmed to have the observed error-function profile (Fig. 2B), where we could control the lapping height H and speed U_{MAX} . The hydrophilic nature of glass (static contact angle = 14°) mimicked the wetness of the tongue. High-speed imaging revealed the formation and extension of a water column, as in lapping (Fig. 3, A to G and movies S2 and S3) (16). The column's ascent was eventually interrupted by pinch-off, leaving a pendantlike drop on the lower surface of the disk (Fig. 3, F to H).

Estimation of the forces involved suggests that the fluid dynamics of lapping are governed by inertia and gravity, whereas viscous and capillary forces are negligible (16). Inertial entrainment draws liquid upward into a column, while gravity acts to collapse it. Ultimately, gravity prevails and the column pinches off. Dimensional analysis reveals that two dimensionless parameters control lapping: the Froude number, $\text{Fr} = U_{\text{MAX}}/(gR)^{1/2}$, measuring the relative importance of inertia and gravity (g is the gravitational acceleration), and the aspect ratio, H/R . Experiments were therefore conducted over a range of Fr and H/R values (16), for a fixed lapping height, $H = 3$ cm, determined from observations (Fig. 2B). The latter is possibly dictated by biological constraints, such as the need to keep whiskers dry to maintain their sensory performance (19) or to maximize peripheral vision while drinking.

To test the proposition that the column dynamics are set by a competition between inertia and gravity, we compared the height of the disk (Z) at pinch-off, Z_p , with that predicted from our scaling analysis. We find (16) that $Z_p/H \sim \text{Fr}^*$ for $\text{Fr}^* < 1$ and $Z_p/H \sim 1$ otherwise. Here, $\text{Fr}^* = (R/H)\text{Fr}^{2/3}$ is the ratio of the time scale for the gravitational collapse of the column, $t_p = (R/U_{\text{MAX}})\text{Fr}^{2/3}$, and the time scale for the upward motion of the disk, H/U_{MAX} . Experiments confirmed the existence of two regimes (Fig. 4A). For small disks ($R = 2.5$ and 5 mm), Z_p/H increased linearly with Fr^* , whereas large disks ($R = 10$ and 12.7 mm)

reached the final height before pinch-off ($Z_p/H = 1$). Theory also successfully predicts that pinch-off occurs close to the disk (Fig. 3).

Consequently, the balance between inertia and gravity dictates the lapping frequency, f , by controlling the time of pinch-off. To maximize ingested volume, which is assumed to be proportional to the column's volume V , the lapping time, $1/f$, should match the pinch-off time, t_p , because faster lapping fails to maximize inertial entrainment, whereas slower lapping results in belated mouth closure that misses most of the column. This predicts $f \sim (gH)^{1/2}/R$ or, because $f \sim U_{\text{MAX}}/H$, that Fr^* is of order one. For domestic cats, we find that Fr^* is indeed of order unity (0.4), using the experimentally measured values $U_{\text{MAX}} = 78$ cm s⁻¹ and $H = 3$ cm (Fig. 2B) and a tongue size of $R \approx 5$ mm (Fig. 1G).

The growth dynamics of the column's volume (Fig. 4B) (16) provide further evidence that the lapping frequency is set by the interplay between inertia and gravity. When the disk is close to the bath ($Z \ll H$), the column is cylindrical and V increases as $V/R^3 = \pi Z/R$ (Fig. 4B inset). Gravity-driven drainage then reduces the rate of volume increase, and V is at a maximum when gravity and inertia balance (Fig. 4B). A scaling argument predicts a maximum volume when the disk height reaches $Z_{\text{MaxVol}}/H \sim \text{Fr}^*$ (16), in excellent agreement with observations (Fig. 4A). This also supports our assumption that V is maximum close to the time of pinch-off. In fact, V always peaks shortly before pinch-off (10 to 70 ms) (Fig. 4B), which suggests that mouth closure should also occur just before pinch-off to maximize captured volume. Observations of domestic cats showed that mouth closure indeed typically preceded pinch-off (movie S1) (16).

Fig. 1. The lapping process. (A to F) Snapshots showing the movement of the tongue of *F. catus* and the dynamics of the liquid column during a lapping cycle. Lapping occurs by fluid adhesion to the dorsal part of the tongue's tip and by lifting a liquid column through the tongue's upward motion, before jaw closure. Time elapsed from the first frame is given in the top left corner of each frame. (G) Photograph of the dorsal side of the tongue of *F. catus*, acquired under anesthesia (16). Only the smooth tip is used in lapping.

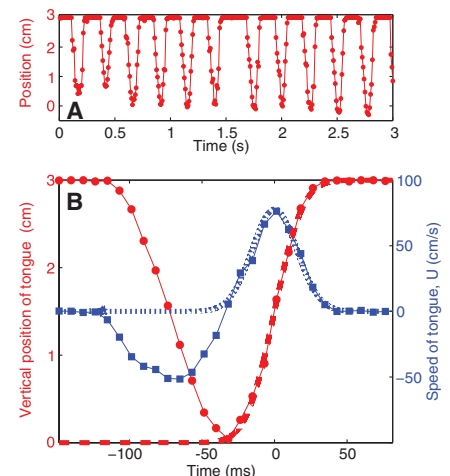
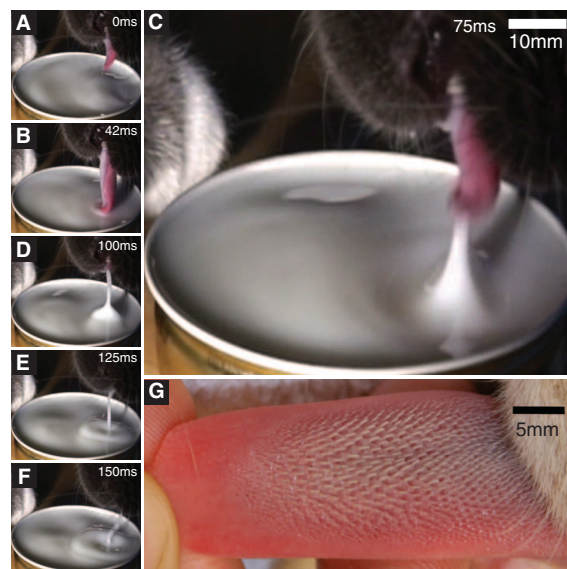


Fig. 2. The lapping kinematics. (A) Vertical position of the tip of the tongue of *F. catus* over 11 lapping cycles. (B) Vertical position (red circles) and velocity (blue squares) of the tongue's tip during a typical lapping cycle, obtained by averaging 11 cycles. The dashed line (red) represents a fitted error-function profile for vertical position. The dotted line (blue) is its temporal derivative and represents upward speed, which yielded $U_{\text{MAX}} = 78 \pm 2$ cm s⁻¹.

Fig. 3. Physical model of lapping. A disk of radius R is driven vertically upward, such that the elevation of the disk, $Z(t)$, from the liquid interface (dashed line, $Z = 0$), has an error-function profile (16). Lowercase z denotes the distance of a generic liquid layer from the disk. The sequence illustrates the formation (A to E) and pinch-off (F) of the liquid column. After pinch-off, part of the column collapses into the bath (G), which leaves a pendantlike drop attached to the disk (H). The final height of the disk is $Z = H$. Time is measured relative to the moment the disk reaches height $Z = H/2 = 1.5$ cm. This sequence corresponds to $R = 12.7$ mm, $H = 3$ cm, $U_{\text{MAX}} = 50$ cm s $^{-1}$ ($Fr = 1.42$; $Fr^* = 0.60$).

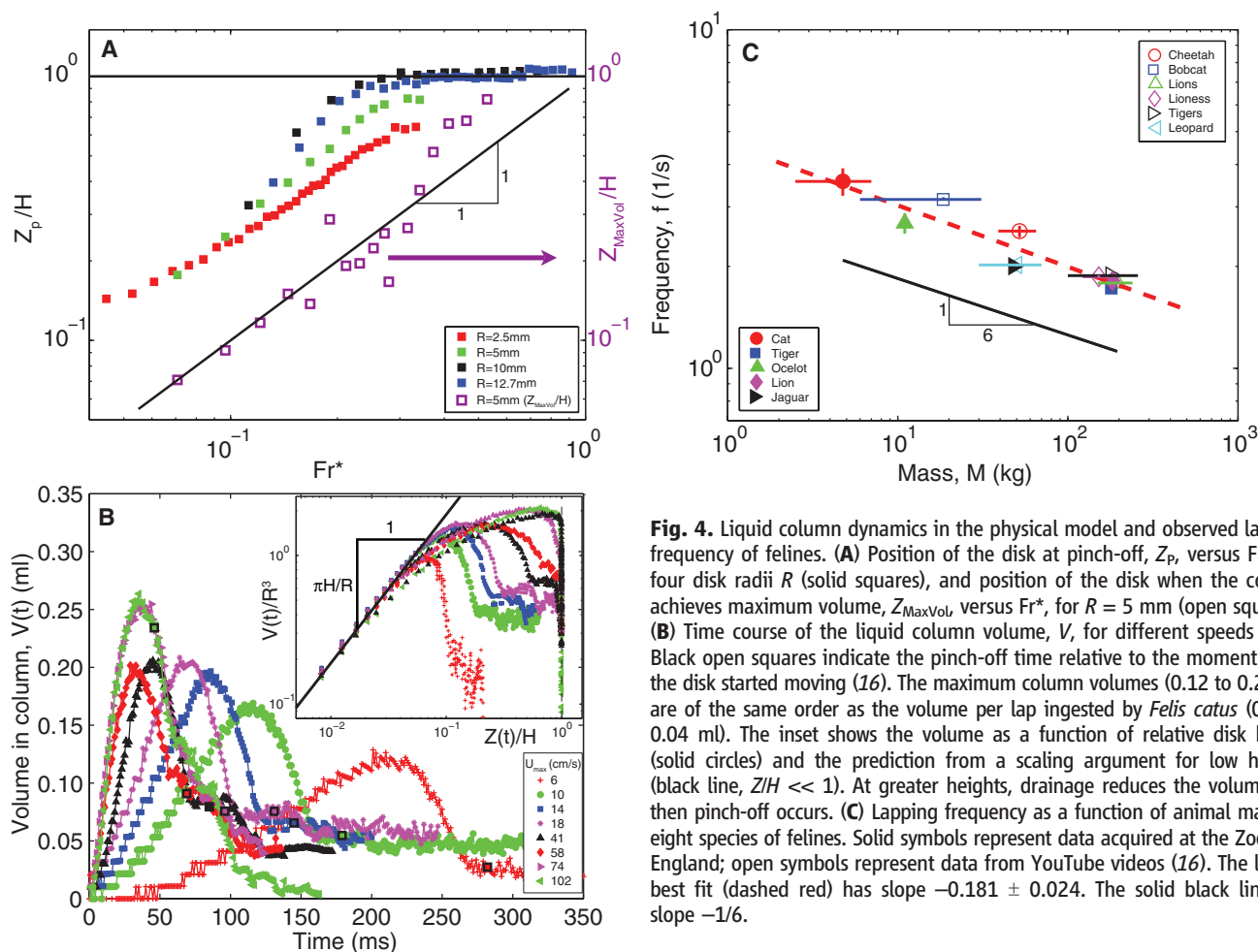
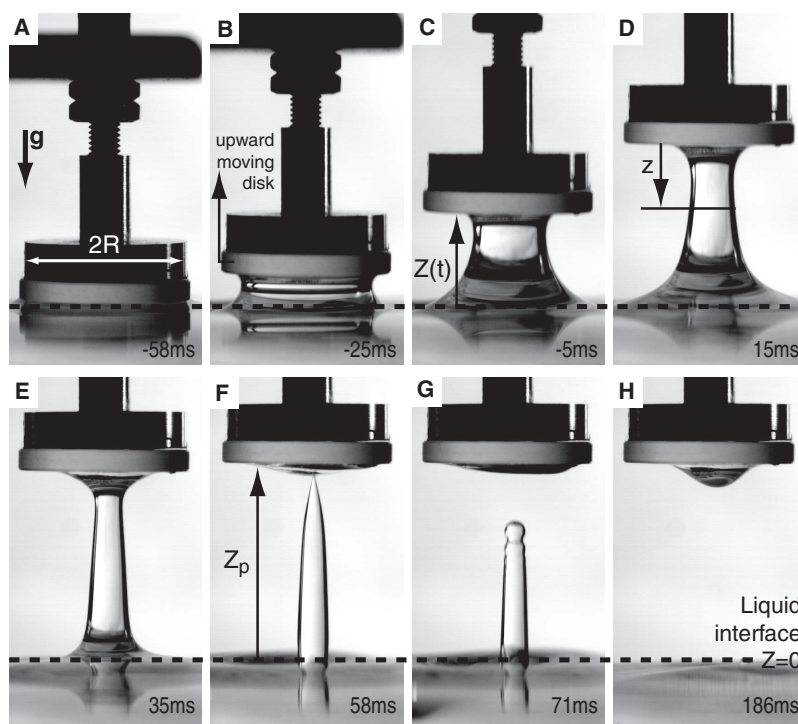


Fig. 4. Liquid column dynamics in the physical model and observed lapping frequency of felines. (A) Position of the disk at pinch-off, Z_p , versus Fr^* , for four disk radii R (solid squares), and position of the disk when the column achieves maximum volume, Z_{MaxVol} , versus Fr^* , for $R = 5$ mm (open squares). (B) Time course of the liquid column volume, V , for different speeds U_{MAX} . Black open squares indicate the pinch-off time relative to the moment when the disk started moving (16). The maximum column volumes (0.12 to 0.26 ml) are of the same order as the volume per lap ingested by *Felis catus* (0.14 ± 0.04 ml). The inset shows the volume as a function of relative disk height (solid circles) and the prediction from a scaling argument for low heights (black line, $Z/H \ll 1$). At greater heights, drainage reduces the volume and then pinch-off occurs. (C) Lapping frequency as a function of animal mass for eight species of felines. Solid symbols represent data acquired at the Zoo New England; open symbols represent data from YouTube videos (16). The line of best fit (dashed red) has slope -0.181 ± 0.024 . The solid black line has slope $-1/6$.

The balance of inertia and gravity yields a prediction for the lapping frequency of other felines. Assuming isometry within the Felidae family (i.e., that lapping height H scales linearly with tongue width R and animal mass M scales as R^3), the finding that Fr^* is of order one translates to the prediction $f \sim R^{-1/2} \sim M^{-1/6}$. Isometry or marginally positive allometry among the Felidae has been demonstrated for skull (20, 21) and limb bones (22). Although variability by function can lead to departures from isometry in interspecific scalings (23), reported variations within the Felidae (23, 24) only minimally affect the predicted scaling $f \sim M^{-1/6}$. We tested this $-1/6$ power-law dependence by measuring the lapping frequency for eight species of felines, from videos acquired at the Zoo New England or available on YouTube (16). The lapping frequency was observed to decrease with animal mass as $f = 4.6 M^{-0.181 \pm 0.024}$ (f in s^{-1} , M in kg) (Fig. 4C), close to the predicted $M^{-1/6}$. This close agreement suggests that the domestic cat's inertia- and gravity-controlled lapping mechanism is conserved among felines.

The lapping of *F. catus* is part of a wider class of problems in biology involving gravity and inertia, sometimes referred to as Froude mechanisms. For example, the water-running ability of the Basilisk lizard depends on the gravity-driven collapse of the air cavity it creates upon slapping the water surface with its feet. The depth to which the lizard's leg penetrates the surface depends on the Froude number, which in turn prescribes the minimum slapping frequency (25). The Froude number is also relevant to swimming, for example, setting the maximum practical swimming speed in ducks (26), and to terrestrial legged locomotion. In this respect, it is interesting to note that the transition from trot to gallop obeys nearly the

same scaling of frequency with mass as lapping, $f = 4.5 M^{-0.14}$ (f in s^{-1} , M in kg) (27).

The subtle use of the tongue in the drinking process of *F. catus* is remarkable, given the tongue's lack of skeletal support (28). Complex movement in the absence of rigid components is a common feature of muscular hydrostats, which in addition to tongues include elephant trunks and octopus arms (28, 29). The functional diversity and high compliance of these structures continue to inspire the design of soft robots (29), and a fundamental understanding of their functionality can lead to new design concepts and is essential to inform biomechanical models (29, 30).

References and Notes

- P. J. Bentley, T. Yorio, *J. Exp. Biol.* **79**, 41 (1979).
- D. Attenborough, *The Living Planet: A Portrait of the Earth* (Collins and British Broadcasting Corporation, London, 1984).
- J. Heidweiller, J. A. van Loon, G. A. Zweers, *Zoomorphology* **111**, 141 (1992).
- J. A. W. M. Weijnen, *Neurosci. Biobehav. Rev.* **22**, 751 (1998).
- W. C. Diller, in *Roots of Behavior*, E. L. Bliss, Ed. (Harper, New York, 1962), pp. 35–47.
- M. Prakash, D. Quéiré, J. W. M. Bush, *Science* **320**, 931 (2008).
- S. W. S. Gussekloo, R. G. Bout, *J. Exp. Biol.* **208**, 3395 (2005).
- T. L. Daniel, J. G. Kingsolver, E. Meyhöfer, *Oecologia* **79**, 66 (1989).
- J. G. Kingsolver, T. L. Daniel, *Oecologia* **60**, 214 (1983).
- D. Cundall, *J. Exp. Biol.* **203**, 2171 (2000).
- J. Heidweiller, G. A. Zweers, *Condor* **92**, 1 (1990).
- J. G. M. Kooloos, G. A. Zweers, *J. Morphol.* **199**, 327 (1989).
- A. J. Thexton, A. W. Crompton, R. Z. German, *J. Exp. Zool.* **280**, 327 (1998).
- K. M. Hiiemae, A. W. Crompton, in *Functional Vertebrate Morphology*, M. Hildebrand, D. Bramble, K. Liem, D. B. Wake, Eds. (Belknap of Harvard Univ. Press, Cambridge, MA, 1985), pp. 262–290.
- A. J. Thexton, J. D. McGarrick, *Arch. Oral Biol.* **33**, 331 (1988).
- Materials and methods are available as supporting material on Science Online.
- Quicker 'n a Wink (Metro-Goldwyn-Mayer Studios, Los Angeles, CA, 1940); www.youtube.com/watch?v=jUZwFrGzQGw.
- K. Ojima, F. Mitsuhashi, M. Nasu, Y. Suzuki, *Ann. Anat.* **182**, 47 (2000).
- A. S. Ahl, *Vet. Res. Commun.* **10**, 245 (1986).
- P. Christiansen, J. S. Adolfsen, *J. Zool. (London)* **266**, 133 (2005).
- A. P. Russell, H. N. Bryant, G. L. Powell, R. Laroia, *J. Zool. (London)* **236**, 161 (1995).
- J. Meachen-Samuels, B. Van Valkenburgh, *J. Morphol.* **270**, 729 (2009).
- M. Doube, A. Wiktorowicz-Conroy, P. Christiansen, J. R. Hutchinson, S. Shefelbine, *PLoS ONE* **4**, e4742 (2009).
- L. M. Day, B. C. Jayne, *J. Exp. Biol.* **210**, 642 (2007).
- J. W. Glasheen, T. A. McMahon, *J. Exp. Biol.* **199**, 2611 (1996).
- H. D. Prange, K. Schmidt-Nielsen, *J. Exp. Biol.* **53**, 763 (1970).
- N. C. Heglund, C. R. Taylor, T. A. McMahon, *Science* **186**, 1112 (1974).
- K. K. Smith, W. M. Kier, *Am. Sci.* **77**, 28 (1989).
- D. Trivedi, C. D. Rahn, W. M. Kier, I. D. Walker, *Appl. Bionics Biomech.* **5**, 99 (2008).
- H. J. Chiel, P. Crago, J. M. Mansour, K. Hathi, *Biol. Cybern.* **67**, 403 (1992).
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Supporting Online Material

www.sciencemag.org/cgi/content/full/330/6008/1231/DC1
Materials and Methods
References
Movies S1 to S3

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Reducing the Gender Achievement Gap in College Science: A Classroom Study of Values Affirmation

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In many science, technology, engineering, and mathematics disciplines, women are outperformed by men in test scores, jeopardizing their success in science-oriented courses and careers. The current study tested the effectiveness of a psychological intervention, called values affirmation, in reducing the gender achievement gap in a college-level introductory physics class. In this randomized double-blind study, 399 students either wrote about their most important values or not, twice at the beginning of the 15-week course. Values affirmation reduced the male-female performance and learning difference substantially and elevated women's modal grades from the C to B range. Benefits were strongest for women who tended to endorse the stereotype that men do better than women in physics. A brief psychological intervention may be a promising way to address the gender gap in science performance and learning.

The substantial underrepresentation of women in science, technology, engineering, and mathematics (STEM) disciplines has long

concerned policy-makers and the educational community (1, 2). In 2006, women earned only 28% of Ph.D.s in physical sciences, 25% in mathematics

and computer science, and 20% in engineering in the United States (3). Although women made up 47% of the North American workforce in 2009, the percentage of women in lucrative technical professions, such as “computer and mathematical occupations” and “architecture and engineering occupations,” reached only 25% and 14%, respectively (4). Similar underrepresentation of women in STEM-related professions is also evident in other parts of the world (5).

The gender gap in STEM disciplines goes beyond the limited representation of women. In college physics—the field studied in the present investigation—women earn lower exam grades and lower scores on standardized tests of conceptual mastery (6, 7). Students' prior background and preparation in mathematics and physics, iden-

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tified as a major contributor to performance in introductory physics (8), can account for as much as 60% of the gender gap in exam performance at our institution, the University of Colorado, but background and preparation do not fully account for the gap (9).

Because gender achievement gaps have important educational and societal implications, several attempts have been made to reduce them. For example, in physics, interactive techniques such as peer instruction (10), where students discuss the answers to conceptual questions in small groups during lectures, and curricular materials, such as tutorials in introductory physics (11) and context-rich problems (12), can reduce the gender gap in college physics classrooms (13). Larger-scale attempts to reduce the gender gap in physics include restructuring the entire physics course (7, 14, 15) or introducing mentoring programs focused on women (16).

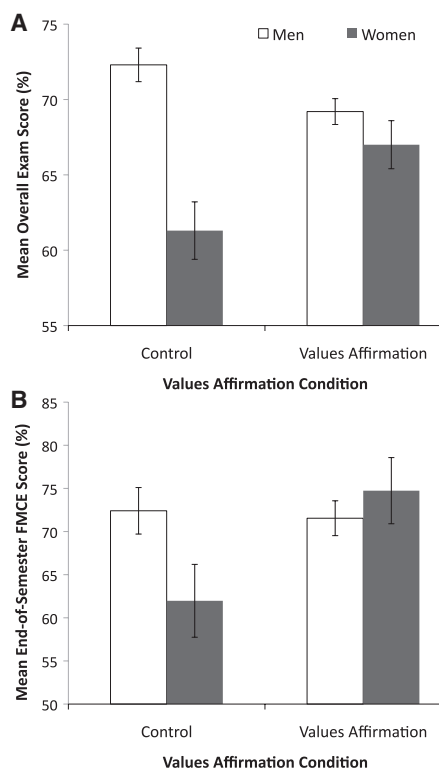


Fig. 1. Student performance on two outcome measures examined in this study as a function of gender (men versus women) and affirmation condition (values affirmation versus control). **(A)** The overall combined exam scores, derived by averaging the percent correct for the three midterm exams (weeks 5, 9, and 14) and the final exam for each student. **(B)** The end-of-semester (week 15) score of the FMCE, a standardized test of conceptual knowledge in physics (33). This test was administered twice (in weeks 1 and 15) in recitation sections to assess the learning of basic physics concepts over the course of the semester. Exam scores are adjusted based on baseline math performance (SAT/ACT Math scores), and the end-of-semester FMCE scores are adjusted based on beginning-of-semester FMCE scores (SOM text). Error bars represent ± 1 SE.

Although some of these attempts have reduced gender gaps, interventions in science education have focused mostly on instructional methods. Many have not taken into account social-psychological and cognitive processes that lead to gender differences in performance and learning. One such process involves psychological threat tied to the groups with which a person identifies. The fear of being devalued based on a group identity, such as becoming aware that one could be seen in light of a negative stereotype about one's group, has been shown to undermine performance on difficult tests (17). For example, women's performance on difficult math and science tests can suffer insofar as they worry that their poor performance could be seen to confirm a negative gender stereotype (18, 19). Although such identity threat has been shown to affect performance in lab experiments and classrooms (20, 21), attempts to reduce identity threat in authentic classroom contexts have been limited (22, 23).

Here, we report a large-scale classroom study that tested the effectiveness of a theoretically motivated psychological intervention, called values affirmation (24, 25), in reducing the gender gap in exam scores and in a standardized test of conceptual physics. Values affirmation could provide a complementary yet comparatively simple strategy to address gender differences in performance and learning in college science classrooms. A novel feature of this study is that it provides a formal assessment of the degree of student learning over a semester.

Physics is a challenging subject for many college students. Learning the material in lectures and textbooks and demonstrating understanding on exams put substantial pressure on students. Because of the stereotype that men are better than women at math and science (26), such pressure can prove more severe for women than men, especially for women who think that the stereotype might be valid and worry that it could apply or be applied to them (20, 27). Values affirmation, in

which people reflect on self-defining values, can buffer people against such psychological threat. When they affirm their core values in a threatening environment, people reestablish a perception of personal integrity and worth, which in turn can provide them with the internal resources needed for coping effectively (24, 25, 28). Indeed, lab studies show that such affirmations lessen evaluative stress (29) and improve the performance of stereotype-threatened individuals (30).

The values-affirmation intervention used in this study involves writing about personally important values (such as friends and family). The writing exercise is brief (10 to 15 min) and is unrelated to the subject matter of the course. Nevertheless, it has been found effective in improving the grades of ethnic minority middle-school students and closing the racial achievement gap (23). Moreover, this benefit persisted in a 2-year follow-up study (31).

In this study, we applied the intervention to an entirely different context: the gender gap in college-level science. We tested whether values affirmation would reduce the gender achievement gap in a 15-week introductory physics course for STEM majors. Because the course had already implemented pedagogical practices aimed at lessening gender gaps (10, 11), this setup provided a strong test of the effectiveness of values affirmation to further reduce the gap. Moreover, compared to the African-American middle-school students in previous classroom intervention studies (23, 31), these students were relatively high-achieving (taking college physics and most planning to be STEM majors) and were from more-advantaged backgrounds.

In this randomized double-blind study, 399 students (283 men and 116 women) were randomly assigned to either the values-affirmation group or the control group (32). Students in the affirmation group selected their most important values from a list (such as relationships with friends and family or learning or gaining knowledge) and, in response to structured prompts, wrote about why

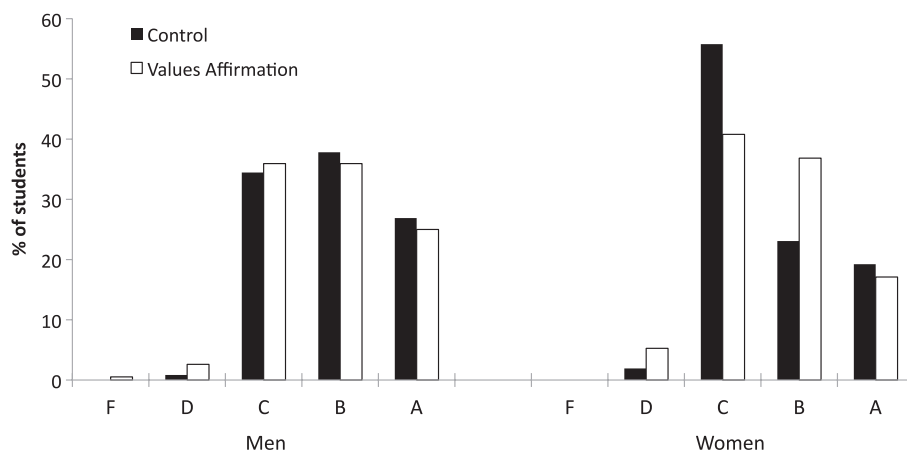


Fig. 2. Percentage of students receiving each letter grade (A, B, C, D, and F, combining letter grades with pluses and minuses) as a function of gender (men versus women) and affirmation condition (values affirmation versus control). The percentage was calculated separately for each values-affirmation condition within each gender.

these values were important to them. Students in the control group selected their least important values from the same list and wrote why these values might be important to other people. Thus, both groups wrote about values and their importance, but the exercise was self-relevant only for the affirmation group. This 15-min writing exercise was integrated into the class and was given once in the first recitation of the semester (week 1) and once in an online homework assignment (week 4) shortly before the first midterm exam (week 5). Each student was assigned to the same condition at both administrations. The course instructor and teaching assistants were unaware of students' condition assignments, and the teaching assistants and students were unaware of the purpose of the writing exercises. As part of an online survey typically given in the course (week 2), students also indicated their endorsement of the stereotype that men perform better than women in physics.

The main outcome measure was scores on in-class exams (three midterms and one final) that consisted entirely of objective multiple-choice questions and constituted 75% of the final course grade. To assess the effect of values affirmation on learning, we also examined scores on an objective, nationally normed standardized test of conceptual physics [the Force and Motion Conceptual Evaluation (FMCE)] (33). Historically, men have substantially outperformed women on exams and the FMCE (9) in this course. In semesters when exams are heavily weighted (as in the semester of the present study), course grades have also shown a gender gap (9).

We predicted a reduced gender gap in performance for women who completed the values affirmation. Moreover, because people who endorse

negative stereotypes about their group are most vulnerable to identity threat (20, 27), we expected the intervention to be particularly beneficial for women tending to endorse the gender stereotype.

The values-affirmation intervention succeeded in reducing the gender gap in performance. As shown in Fig. 1A, men outperformed women in the control condition on overall exam scores (the average of the percent correct for the four exams). However, the gender gap was significantly smaller in the affirmation condition than in the control condition, resulting in a significant gender $\times$ condition interaction [$\beta = 0.16$, $t(387) = 3.08$, $P < 0.01$] (34). The effect size for the observed gender gap was substantial in the control condition (Cohen's $d = 0.93$) ($F_{1,387} = 36.71$, $P < 0.01$) but much smaller in the affirmation condition ($d = 0.18$) ($F_{1,387} = 2.35$, $P = 0.13$). Course grades, based substantially (75%) on the exam scores, showed a similar pattern [supporting online material (SOM) text].

Although the second affirmation exercise was completed shortly before the first midterm exam, its benefits were not confined to that exam. The reduction in the gender gap remained evident on the final cumulative exam (table S1), as indicated by a significant gender $\times$ condition interaction [$\beta = 0.19$, $t(387) = 3.45$, $P < 0.01$; affirmation effect for women, $F_{1,387} = 12.49$, $P < 0.01$], even though no additional values-affirmation exercises took place beyond the fourth week of class.

The distribution of final letter grades indicated that values affirmation was particularly effective in improving women's performance from average (C) to above average (B). As shown in Fig. 2, women in the two groups differed primarily in the B-to-C range; more women earned B's in the affirmation group than in the control group, whereas

more women earned C's in the control group than in the affirmation group [$\chi^2(1, N = 91) = 4.07$, $P = 0.04$]. There was no such difference in grade distribution for men [$\chi^2(1, N = 202) = 0.02$, $P = 0.88$].

The benefit of values affirmation for women was also observed on the FMCE. It was administered twice as part of the course, once at the beginning of the semester (week 1) and once at the end (week 15), to assess learning of physics concepts over the semester. Students were explicitly told that their performance would not influence their grades. Although there was no main or interactive effect of condition on the beginning-of-semester score [t 's < 1 , NS], there was an effect at the end of the semester. Figure 1B illustrates the end-of-semester FMCE scores, controlling for scores on the beginning-of-semester scores to isolate effects on learning. The gender gap in the learning of physics concepts was substantial in the control condition ($d = 0.46$) ($F_{1,304} = 6.23$, $P = 0.01$), indicating that men improved their FMCE scores more than women over the semester. In the affirmation condition, however, this gender learning gap entirely disappeared ($d = -0.12$) ($F_{1,304} = 0.96$, $P = 0.33$), resulting in a significant gender $\times$ condition interaction [$\beta = 0.12$, $t(296) = 2.13$, $P = 0.03$]. That the benefit of affirmation was evidenced on the end-of-semester FMCE scores with beginning-of-semester scores controlled suggests that the intervention facilitated women's learning of scientific concepts over the semester (SOM text).

Unexpectedly, affirmation negatively affected men's exam scores (Fig. 1A), but, unlike the positive effect for women, this effect was not predicted, was not replicated for the end-of-semester FMCE score (Fig. 1B), and did not change men's letter grade distribution (Fig. 2) (35). In contrast, the affirmation's positive effect on women was significant for all outcome variables (SOM text), suggesting that the reduced gender gap observed in this study is based more robustly on the affirmation's positive impact on women than on its negative impact on men.

Finally, the values affirmation was particularly beneficial for women who tended to endorse the gender stereotype. This moderation effect is illustrated in Fig. 3, with the exam (Fig. 3A) and end-of-semester FMCE (Fig. 3B) data. Although women as a group did not strongly endorse the negative gender stereotype (20), even a moderate level of stereotype endorsement was costly for women in the control condition, with their exam scores decreasing as a function of stereotype endorsement [$\beta = -0.50$, $t(387) = -3.29$, $P < 0.01$] (Fig. 3A). Affirmation, however, buffered women against this identity threat, eliminating the negative relation between stereotype endorsement and exam scores [$\beta = 0.12$, $t(387) = 0.94$, $P = 0.35$]. Moreover, among women expressing higher levels of stereotype endorsement (defined as 0.75 SDs above the mean here), affirmation improved the exam scores relative to the control condition [$t(115) = 3.04$, $P < 0.01$]. In contrast, men's exam scores were little affected by stereotype endorsement, regardless of condition [$\beta = -0.08$, $t(387) =$

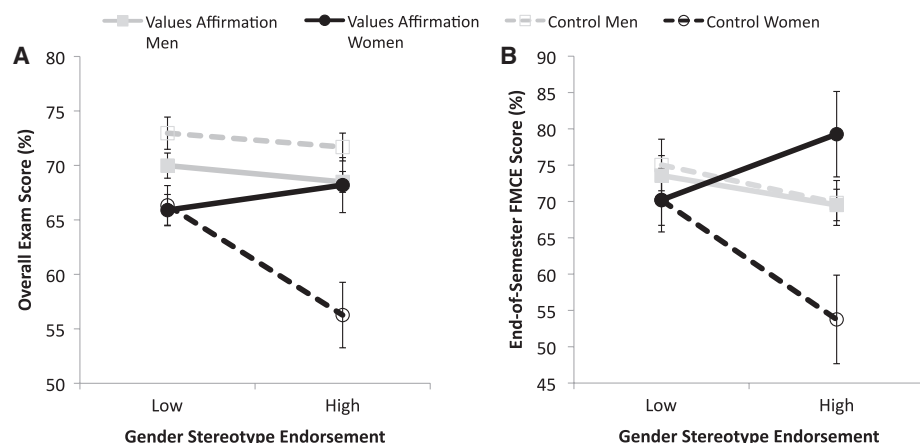


Fig. 3. Student performance on two outcome measures examined in this study as a function of gender (men versus women), affirmation condition (values affirmation versus control), and the level of stereotype endorsement. (A) The overall combined exam scores, computed by averaging the percentage scores for the four exams. (B) The end-of-semester (week 15) score on the FMCE, a standardized test of conceptual knowledge in physics (33). Stereotype endorsement, treated as a continuous variable in our statistical analysis, was measured by agreement with the statement, "According to my own personal beliefs, I expect men to generally do better in physics than women," answered on a five-point scale ranging from "strongly disagree" to "strongly agree." The level of stereotype endorsement plotted in the graph was standardized for all students and expressed in terms of z scores (20). The low and high levels of stereotype endorsement in the graph are represented by ± 0.75 SD of the grand mean (SOM text). Error bars represent ± 1 SE.

-1.70 , $P = 0.09$, for the affirmation condition, and $\beta = -0.07$, $t(387) = -0.92$, $P = 0.36$, for the control condition]. These differential patterns for men and women resulted in a gender $\times$ condition $\times$ stereotype endorsement interaction [$\beta = 0.16$, $t(387) = 2.74$, $P < 0.01$].

A similar three-way interaction was found for the FMCE scores (Fig. 3B) [$\beta = 0.15$, $t(296) = 2.45$, $P = 0.02$]. Among women, there was a negative relationship between stereotype endorsement and end-of-semester FMCE scores in the control condition [$\beta = -0.39$, $t(296) = -2.55$, $P = 0.01$], but not in the affirmation condition [$\beta = 0.22$, $t(296) = 1.54$, $P = 0.13$]. Moreover, among women with higher levels of stereotype endorsement, the end-of-semester FMCE scores were higher in the affirmation condition than in the control condition [$t(115) = 3.01$, $P < 0.01$]. No such relationship was observed for men in either the control condition [$\beta = -0.13$, $t(296) = -1.33$, $P = 0.18$] or the affirmation condition [$\beta = -0.10$, $t(296) = -1.41$, $P = 0.16$].

Overall, these results suggest that values affirmation is a promising intervention that can help reduce the gender achievement gap in physics. Although the intervention was brief and did not directly concern the course material, it nonetheless provided a meaningful boost for women—especially women who tended to endorse the gender stereotype—on two objective measures: in-class multiple-choice exams and a standardized test of conceptual mastery (FMCE). Moreover, the results on the end-of-semester FMCE provide evidence that identity threat and affirmation affect the learning of scientific concepts, not just performance (SOM text). After controlling for prior background (prior SAT/ACT Math or beginning-of-semester FMCE scores), the affirmation closed the “residual” gender gap on in-class exam scores by approximately 61% and entirely eliminated the gap on the FMCE. Although further efforts must aim to close the gap in prior preparation, the current results are promising in demonstrating that a brief psychological intervention can help close the residual gap, a problem of long-standing concern in science (9).

The introductory physics class we investigated was intended for STEM majors who have had success in STEM-related subjects before college and are motivated to do well in the course. Our results, therefore, demonstrate that, even among women who are relatively identified with and accomplished in science, a substantial gender gap exists, women’s performance is negatively related to stereotype endorsement, and gender differences can be reduced with a values-affirmation intervention.

Although previous attempts to reduce the gender achievement gap in science have focused mostly on instructional methods, the current results highlight the importance of social-psychological factors. One virtue of the affirmation is that it can be combined with instructional approaches that show promise in closing the gender gap, such as the interactive engagement approaches used in the present course (10). However, there is no reason

to think that the effects of affirmation are confined to situations in which such instructional supports are already in place, given that the intervention was successful in reducing racial achievement differences among middle-school students in traditional classrooms (23, 31). Of course, even here, there were structural opportunities for learning in the form of a solid curriculum and qualified teachers; without such basic support, the efficacy of any psychological intervention would be limited (23).

Another virtue of the values-affirmation intervention is that it is not tied to a specific discipline. The psychological phenomenon that the intervention targets—identity threat—can prove an obstacle in other STEM disciplines where the underrepresentation and underperformance of women have been evident. Thus, the intervention has potential for broad applicability in many STEM disciplines.

Finally, the benefits of the affirmation may be long-lasting (25, 31) and could persist beyond the present course. The experience of lower identity threat, coupled with better mastery in a challenging science course, may encourage affirmed women to take other STEM courses and to pursue further education and even a career in STEM disciplines. More generally, the cumulative consequences of early performance—small differences at an early stage can get magnified over time—help explain how relatively brief interventions, when given early in a threatening environment, can have long-term effects. This snowballing effect may be particularly important in science, where subsequent learning builds on an earlier foundation of knowledge, making it increasingly difficult to catch up and enter a discipline later. Therefore, it may be important to intervene in gateway courses such as introductory physics. These courses are required for STEM majors, and performance in them can set long-term academic and career trajectories. Reducing the gender gap at gateways could not only benefit women’s performance in the short term but also encourage them to choose and persist in a scientific major and career path in STEM disciplines.

References and Notes

- National Academy of Sciences, *Rising Above the Gathering Storm: Energizing and Employing America for a Brighter Future* (National Academy Press, Washington, DC, 2005).
- National Science Board, *The Science and Engineering Workforce: Realizing America’s Potential* [NSF03–69, National Science Foundation (NSF), Arlington, VA, 2003].
- NSF, Division of Science Resources Statistics, *Science and Engineering Degrees: 1966–2006* (detailed statistical tables in NSF 08–321, NSF, Arlington, VA, 2008); available at www.nsf.gov/statistics/nsf08321/.
- U.S. Bureau of Labor Statistics (2009); available at [ftp://ftp.bls.gov/pub/special.requests/lfaaat11.txt](http://ftp.bls.gov/pub/special.requests/lfaaat11.txt).
- Anita Borg Institute for Women and Technology, *The State of Women and Technology Fields Around the World*; available at <http://anitaborg.org/files/womenhightechworld.pdf>.
- S. J. Pollock, N. D. Finkelstein, L. E. Kost, *Phys. Rev. Spec. Top. Phys. Ed. Res.* **3**, 010107 (2007).
- E. Brewster et al., *Phys. Rev. Spec. Top. Phys. Ed. Res.* **6**, 010106 (2010).
- Z. Hazari, R. H. Tai, P. M. Sadler, *Sci. Ed.* **91**, 847 (2007).
- L. E. Kost, S. J. Pollock, N. D. Finkelstein, *Phys. Rev. Spec. Top. Phys. Ed. Res.* **5**, 010101 (2009).
- E. Mazur, *Peer Instruction: A User’s Manual* (Prentice-Hall, Upper Saddle River, NJ, 1997).
- L. C. McDermott, P. S. Schaffer, *Tutorials in Introductory Physics* (Prentice-Hall, Upper Saddle River, NJ, 2002).
- P. Heller, R. Keith, S. Anderson, *Am. J. Phys.* **60**, 627 (1992).
- M. Lorenzo, C. Crouch, E. Mazur, *Am. J. Phys.* **74**, 118 (2006).
- P. Laws, P. Rosborough, F. Poody, *Am. J. Phys.* **67**, S32 (1999).
- S. Brahmia, E. Etkina, *J. Coll. Sci. Teach.* **31**, 183 (2001).
- M. Schneider, *Phys. Teach.* **39**, 280 (2001).
- C. M. Steele, S. J. Spencer, J. Aronson, in *Advances in Experimental Social Psychology*, M. P. Zanna, Ed. (Academic Press, San Diego, CA, 2002), pp. 183–242.
- S. J. Spencer, C. M. Steele, D. M. Quinn, *J. Exp. Soc. Psychol.* **35**, 4 (1999).
- S. L. Beilock, R. J. Rydell, A. R. McConnell, *J. Exp. Psychol. Gen.* **136**, 256 (2007).
- T. Schmader, M. Johns, M. Barquissau, *Sex Roles* **50**, 835 (2004).
- T. Schmader, M. Johns, C. Forbes, *Psychol. Rev.* **115**, 336 (2008).
- C. Good, J. Aronson, M. Inzlicht, *Appl. Dev. Psychol.* **24**, 645 (2003).
- G. L. Cohen, J. Garcia, N. Apfel, A. Master, *Science* **313**, 1307 (2006).
- C. M. Steele, in *Advances in Experimental Social Psychology*, L. Berkowitz, Ed. (Academic Press, New York, 1988), pp. 261–302.
- G. L. Cohen, J. Garcia, *Curr. Dir. Psychol. Sci.* **17**, 365 (2008).
- L. L. Schiebinger, *Has Feminism Changed Science?* (Harvard Univ. Press, Cambridge, MA, 2001).
- J. Aronson, M. Inzlicht, *Psychol. Sci.* **15**, 829 (2004).
- D. K. Sherman, G. L. Cohen, in *Advances in Experimental Social Psychology*, M. P. Zanna Ed. (Academic Press, San Diego, CA, 2006), pp. 183–242.
- J. D. Creswell et al., *Psychol. Sci.* **16**, 846 (2005).
- A. Martens, M. Johns, J. Greenberg, J. Schimmel, *J. Exp. Soc. Psychol.* **42**, 236 (2006).
- G. L. Cohen, J. Garcia, V. Purdie-Vaughns, N. Apfel, P. Brzustoski, *Science* **324**, 400 (2009).
- Materials and methods are available as supporting material on Science Online.
- R. K. Thornton, D. R. Sokoloff, *Am. J. Phys.* **66**, 338 (2006).
- The effect of values affirmation on exam scores and end-of-semester FMCE scores was tested in regression models in which the outcome measures were regressed on gender, affirmation condition, and the gender $\times$ condition interaction. SAT/ACT Math scores were controlled for in the analysis of exam scores, and beginning-of-semester FMCE scores were controlled for in the analysis of end-of-semester FMCE scores. All β weights reported in this article are standardized weights. The full regression models are described in the SOM.
- This unexpected finding for men’s in-class exam scores is discussed in more detail in the SOM. In addition to the mixed evidence summarized here, this negative affirmation effect on men’s exam scores was not significant when the analysis was conducted with the beginning-of-semester FMCE scores (instead of SAT/ACT Math scores) as the covariate (S22).
- We are grateful to course instructor M. Dubson, the course teaching assistants, and the student participants. We also thank L. Newnes and N. Golaszewski for invaluable assistance with data collection and C. Judd and B. Park for their statistical advice. This research was supported by NSF grant DRL0910373.

Supporting Online Material

www.sciencemag.org/cgi/content/full/330/6008/1234/DC1

Materials and Methods
SOM Text
Table S1
References and Notes

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Lynx1, a Cholinergic Brake, Limits Plasticity in Adult Visual Cortex

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Experience-dependent brain plasticity typically declines after an early critical period during which circuits are established. Loss of plasticity with closure of the critical period limits improvement of function in adulthood, but the mechanisms that change the brain's plasticity remain poorly understood. Here, we identified an increase in expression of Lynx1 protein in mice that prevented plasticity in the primary visual cortex late in life. Removal of this molecular brake enhanced nicotinic acetylcholine receptor signaling. Lynx1 expression thus maintains stability of mature cortical networks in the presence of cholinergic innervation. The results suggest that modulating the balance between excitatory and inhibitory circuits reactivates visual plasticity and may present a therapeutic target.

The waxing and waning of cortical plasticity during a postnatal critical period serves to consolidate neural circuits and behavior (1), but in turn limits recovery of function in the adult brain (2). For example, discordant vision through the two eyes during an early critical period results in the enduring loss of visual acuity (amblyopia) that reflects aberrant circuit remodeling within primary visual cortex (V1). Amblyopia, which affects 2 to 4% of the human population, exhibits little recovery in adulthood (3). Identifying specific biological mechanisms that restrict adult plasticity would inspire potentially novel strategies for therapy.

We hypothesized that the gradual emergence of molecular “brakes” might actively prevent plasticity in the adult brain. The only molecules previously reported to play a role in closing the critical period are related to axonal growth inhibition, such as chondroitin sulfate proteoglycans and the myelin-signaling proteins NgR and PirB (4–6). To identify further targets, we analyzed the transcriptome of the binocular zone in mouse V1 for molecules that are expressed more in adulthood than during the critical period (7). Here, we characterize one of these, Lynx1, which is an endogenous prototoxin similar to α -bungarotoxin in snake venom and binds to the nicotinic acetylcholine receptor (nAChR) (8).

Lynx1 expression increases only after the critical period for amblyopia in adult V1 both at the protein and mRNA level (Fig. 1A). Along the visual pathway, *Lynx1* transcripts were expressed both in V1 and the lateral geniculate nucleus (LGN) (Fig. 1B). In contrast, expression of another member of the lynx family, *Lynx2*, declined over the critical period and was hardly found in the visual pathway (fig. S1). We therefore directly assessed

Lynx1 function in the binocular region by electrophysiological recordings from knockout mice.

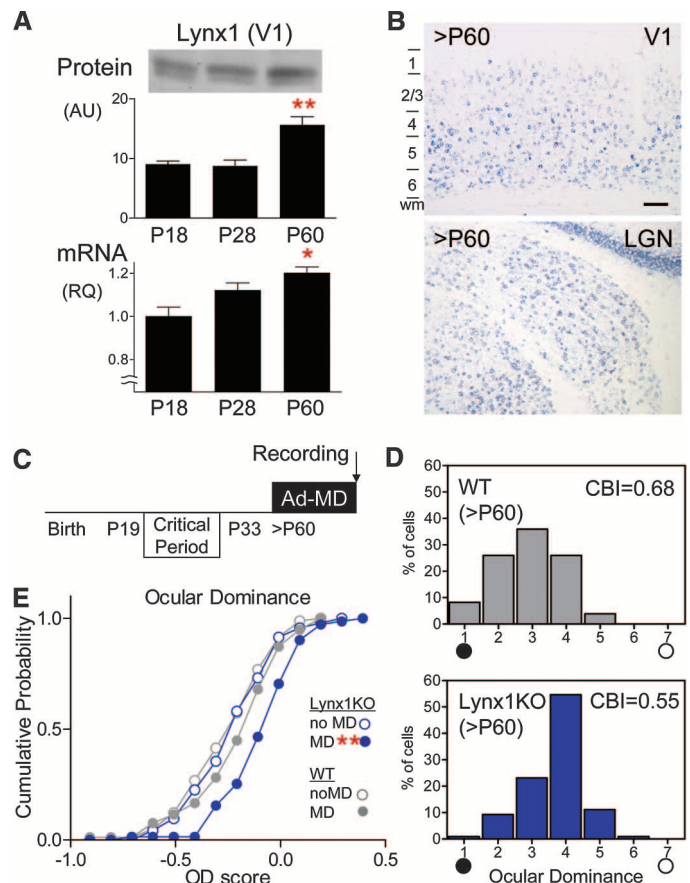
In mice lacking the *Lynx1* gene, the eye preference of single neurons (ocular dominance) was no different from that of wild-type mice (Fig. 1E). Upon short-term (4 day) monocular deprivation (MD) in mature wild-type animals (>postnatal day 60, P60), there was little change in the visual spiking response (3). Instead, adult Lynx1 knockout mice exhibited a robust shift in responsiveness away from the deprived eye (Fig. 1, C to E). This heightened plasticity was specific to

older ages, because short-term MD was equally effective in both wild-type and Lynx1 knockout mice during the critical period (Fig. 2A).

Lynx1 protein directly binds to nAChRs (9), such as the major central subunits $\alpha_4\beta_2$ heteromers or α_7 homomers, to reduce their sensitivity to acetylcholine. We directly assessed the response to systemic nicotine injection in Lynx1 knockout mice by measuring visual evoked potential (VEP) response in anesthetized V1. Enhancement of the VEP response was only observed in Lynx1 knockout mice (Fig. 2, B and C). To test whether nAChR signaling mediates adult plasticity in Lynx1 knockout mice, we applied the broad-spectrum antagonist mecamylamine concurrent with short-term MD. Either systemic injection or restricted infusion directly into V1 by osmotic minipump was sufficient to prevent adult plasticity. These results were corroborated by systemic treatment with a mixture of α_4 - and α_7 -subunit-selective nAChR antagonists (10), dihydro- β -erythroidine (DH β E) plus methyllycaconitine (MLA) (Fig. 2A).

To establish the clinical relevance of these findings, we directly measured recovery from amblyopia in adulthood. In wild-type mice, long-term MD spanning the entire critical period results in a significant reduction of visual acuity as measured directly in V1 by VEP (3). Notably, this reduction persisted into adulthood even if the closed eye was

Fig. 1. Lynx1 expression increases in adulthood to limit visual plasticity. (A) Expression of Lynx1 protein (top) and mRNA (bottom) across the critical period (CP) (pre-CP: P18; CP: P28; post-CP: P60). ** $P < 0.01$, * $P < 0.05$, one-way analysis of variance. AU, arbitrary units; RQ, relative quantification. Data are shown as the mean $\pm$ SEM. (B) In situ hybridization of *Lynx1* in adult V1 (top) and LGN (bottom). Scale bar, 100 μ m. (C) Adult V1 plasticity paradigm by short-term MD (Ad-MD). (D) Ad-MD shifts the ocular dominance distribution of Lynx1 knockout (KO) mice [bottom; contralateral bias index (CBI) = 0.55, 216 cells, 8 mice], but not in wild-type (WT) mice (top; CBI = 0.68, 231 cells, 9 mice). KO versus WT: $P < 0.0001$, χ^2 test. (E) Cumulative probability of quantified spike response after Ad-MD confirms shifted ocular dominance scores for Lynx1 KO (blue filled circles), compared to WT (gray filled circles) (** $P < 0.005$, Kolmogorov-Smirnov test) or no MD (blue open circles, KO, 93 cells; gray open circles, WT, 82 cells; $P = 0.75$, Kolmogorov-Smirnov test).



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reopened for more than 1 month after the critical period (Fig. 3, A and C). Lynx1 knockout mice spontaneously recovered visual acuity to normal

levels simply by reopening the closed eye (Fig. 3, A and C), exhibiting VEPs even at higher spatial frequencies (Fig. 3B). Given the cholinergic basis

Fig. 2. Nicotinic receptors mediate adult plasticity in Lynx1 KO mice. **(A)** Mice without MD (open circles, gray WT/blue KO) shift equally after MD during the CP (light blue, KO: mean CBI = 0.48, 6 mice; light gray, WT: CBI = 0.50, 8 mice; $P > 0.5$, t test). Adult plasticity (blue, KOMD: CBI = 0.55, 12 mice versus gray, WTMD: CBI = 0.68, 9 mice; $***P < 0.0001$, t test) is abolished by concurrent nAChR antagonists (red, KOMD + mecamylamine: CBI = 0.68, 9 mice versus KOMD, $***P < 0.0001$; versus gray, WTMD + mecamylamine: CBI = 0.69, 4 mice, $P > 0.7$; versus no MD KO + mecamylamine: CBI = 0.68, 7 mice, $P > 0.9$, t test; orange, KOMD + DH β E/MLA: CBI = 0.68, 7 mice versus KOMD, $***P < 0.0001$, t test). Darker circles represent cortical minipump infusion. **(B)** Enhanced nicotine response in Lynx1 KO mice. Averaged VEP traces (mean $\pm$ SEM) before (light gray) and 10 min after (black) subcutaneous nicotine injection (+nic) in WT (left) and Lynx1 KO mice (right). **(C)** Integrated VEP (area of first negative peak) for WT (empty bars, 6 mice) and Lynx1 KO mice (filled bars, 11 mice). $*P < 0.05$, t test; n.s., not significant.

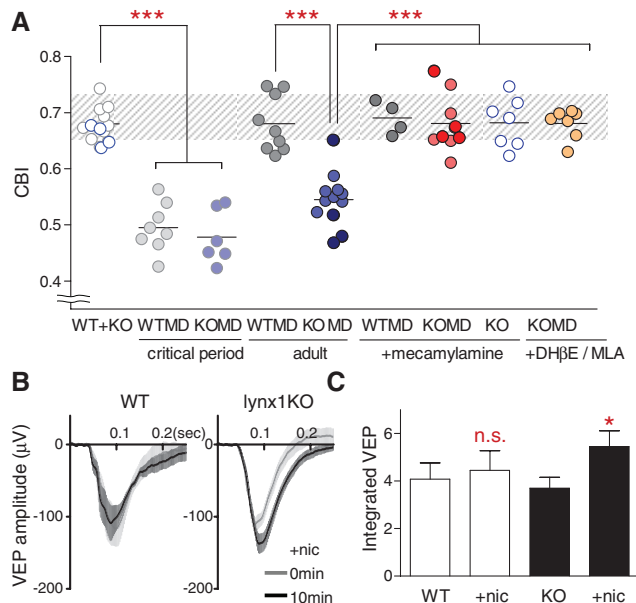
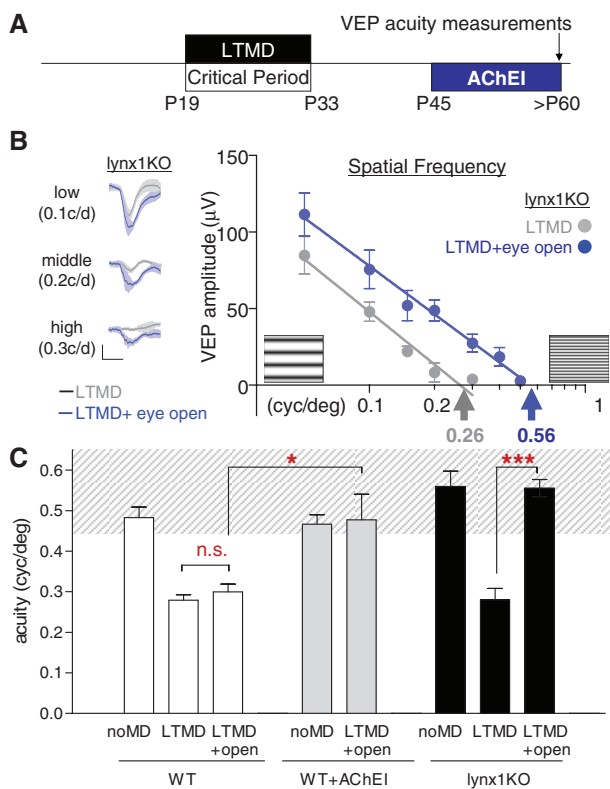


Fig. 3. Recovery from amblyopia in Lynx1 KO mice. **(A)** After long-term MD (LTMD) spanning the critical period (P19 to P33), the deprived eye was reopened (>1 month) until VEP acuity was measured in V1 (>P60). For AChEi experiments, physostigmine was injected daily starting at P45. **(B)** Averaged VEP traces (left, mean $\pm$ SEM; scale: 20 μ V, 0.1 s) and amplitudes (right) of first negative peak (mean $\pm$ SEM) reveal acuity recovery after reopening an eye (blue, 6 mice) initially deprived during the critical period (gray, 5 mice). **(C)** Visual acuity in WT mice (white bars) without deprivation [no MD: 0.48 ± 0.03 cycles per degree (cyc/deg), 6 mice] decreases after LTMD (LTMD: 0.28 ± 0.01 cyc/deg, 3 mice) and endures (+eye open: 0.30 ± 0.02 cyc/deg, 5 mice; versus LTMD, $P > 0.45$; versus no MD, $P < 0.0005$, t test). In contrast, reopening the deprived eye together with cholinesterase inhibitor restores vision (gray bar, AChEi: 0.48 ± 0.06 cyc/deg, 4 mice; versus WT + eye open, $*P < 0.05$; versus WT no MD + AChEi: 0.47 ± 0.02 cyc/deg, 6 mice, $P > 0.8$, t test). Lynx1 KO mice (black bars) spontaneously recover from LTMD (0.28 ± 0.03 cyc/deg, 5 mice) simply by reopening the deprived eye (0.56 ± 0.02 cyc/deg, 6 mice; $***P < 0.0001$, t test) to reach normal levels (no MD: 0.56 ± 0.04 cyc/deg, 3 mice).



of this plasticity, we further attempted to induce recovery even in adult wild-type mice by enhancing endogenous ACh signaling. Injection of an acetylcholinesterase inhibitor, physostigmine, during the period of eye reopening similarly restored vision to wild-type mice initially rendered amblyopic (Fig. 3C).

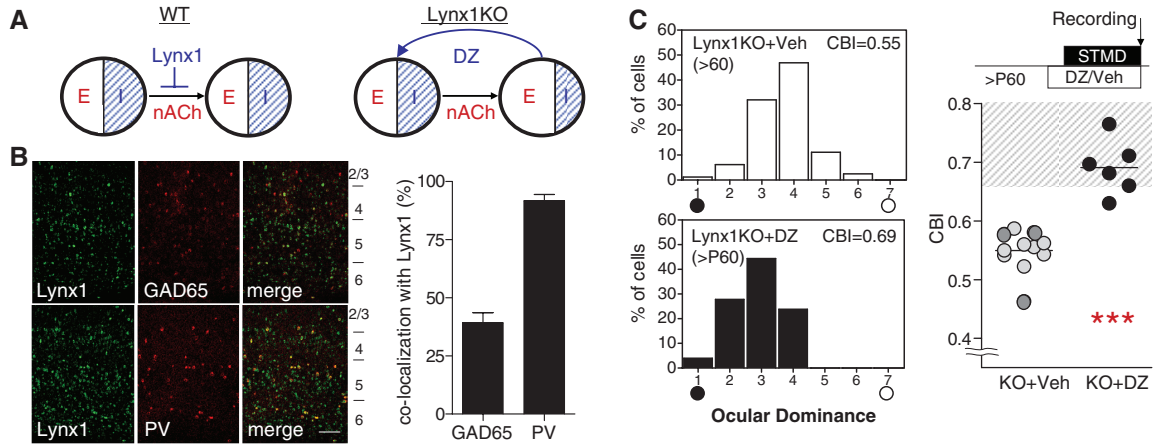
Recovery of function in Lynx1 knockout mice is likely due to an enhanced visual responsiveness during arousal. We did not observe structural changes at the level of perineuronal nets (4) or myelination (5) in Lynx1 knockout mice (fig. S2). Aging-related neurodegeneration reported previously in these animals (11) was confirmed to occur only past 9 months of age. Instead, local excitatory-inhibitory circuit balance might have been affected earlier (3) (Fig. 4A). Previous reports across various species have localized nAChRs to thalamocortical terminals presynaptic to principal cells (12–16), facilitating excitation in V1 (17, 18). Activation of nAChRs upon specific inhibitory neurons could further modulate excitatory-inhibitory balance by disinhibition (19, 20), as in the case of congenital nAChR mutation that disrupts GABA-mediated transmission (21).

Indeed, Lynx1 and nAChR mRNAs were coexpressed not only in the LGN (fig. S3) but also in a subpopulation of GABA cells, primarily parvalbumin-positive interneurons (Fig. 4B and fig. S3B). Activation of nAChRs may also exert long-term epigenetic effects on GABA synthesis (22). To probe whether excitatory-inhibitory imbalance may contribute to adult plasticity in Lynx1 knockout mice, we directly restored intracortical inhibition by focal benzodiazepine infusion from osmotic minipumps. Diazepam treatment of V1 abolished adult plasticity in Lynx1 knockout mice (Fig. 4C), as did nAChR blockade (Fig. 2A). Thus, Lynx1 reduces adult plasticity through cholinergic signaling mechanisms that may adjust excitatory-inhibitory balance later in life (3).

Taken together, Lynx1 provides both a valuable endogenous tool with which to probe critical-period closure and offers novel therapeutic and conceptual insight. In contrast to muscarinic receptors engaged during the critical period (23), our results highlight a nicotinic component for adult V1 plasticity. Although we do not rule out a role for muscarinic receptors (24), deletion of Lynx1 alone is sufficient to rescue visual acuity. Recovery strategies aimed at the Lynx1-nAChR interaction (8, 9) could be fruitful in conjunction with attentional tasks that stimulate cholinergic release (e.g., perceptual learning, video-game training) (25–28). Clinically approved cholinesterase inhibitors that boost the afferent response in human visual cortex (29) may be useful for treating some amblyopes (Fig. 3C), including those with subcortical changes (30). Amblyopia might further serve as a diagnostic measure to identify tobacco exposure (31) or schizophrenia (32).

Although a permissive role for cholinergic input has long been appreciated during the critical period (33), it has remained a mystery why V1 plasticity is severely restricted in adulthood even in the presence of massive innervation from the basal forebrain. Lynx1 expression not only

Fig. 4. Lynx1 may adjust cortical excitatory-inhibitory balance to regulate adult plasticity. **(A)** In WT animals (left), mature excitatory-inhibitory balance is maintained by Lynx1 that limits nAChR response. In Lynx1 KO mice (right), enhanced nAChR signaling may lead to excitatory-inhibitory imbalance and adult plasticity, which could be sensitive to acute restoration of inhibition with diazepam (DZ). **(B)** Double in situ hybridization of Lynx1 (green) with GAD65 (red, top) or parvalbumin (PV, bottom) in adult V1 (left). Scale bar, 100 μ m. Quantification of overlapping pixels (right) indicates selective expression of Lynx1 in a subset (40%) of GAD65-positive interneurons, most likely PV-positive cells (>90% colocalization). **(C)**



Focal diazepam infusion during adult MD in Lynx1 KO mice abolishes ocular dominance plasticity (black, DZ: CBI = 0.67, 6 mice versus gray, vehicle (Veh): CBI = 0.54, 14 mice; ***P < 0.001, t test). Dark circles represent cortical minipump infusion.

contributes to nAChR agonist binding and desensitization kinetics (8), but also may respond to changes in network activity (34). Local regulation of Lynx1 levels may allow cholinergic activation to induce islands of plasticity while maintaining overall circuit stability. Visual attention tasks in fact preferentially modulate fast-spiking inhibitory neurons (35, 36), consistent with a convergence of top-down influences upon local excitatory-inhibitory circuit balance.

References and Notes

1. T. K. Hensch, *Annu. Rev. Neurosci.* **27**, 549 (2004).
2. B. A. Wandell, S. M. Smirnakis, *Nat. Rev. Neurosci.* **10**, 873 (2009).
3. H. Morishita, T. K. Hensch, *Curr. Opin. Neurobiol.* **18**, 101 (2008).
4. T. Pizzorusso et al., *Science* **298**, 1248 (2002).
5. A. W. McGee, Y. Yang, Q. S. Fischer, N. W. Daw, S. M. Strittmatter, *Science* **309**, 2222 (2005).
6. J. Syken, T. Grandpre, P. O. Kanold, C. J. Shatz, *Science* **313**, 1795 (2006).
7. C. Plessy et al., *PLoS ONE* **3**, e3012 (2008).
8. J. M. Miwa et al., *Neuron* **23**, 105 (1999).
9. I. Ibañez-Tallon et al., *Neuron* **33**, 893 (2002).
10. J. A. Davis, T. J. Gould, *Psychopharmacology (Berl.)* **184**, 345 (2006).
11. J. M. Miwa et al., *Neuron* **51**, 587 (2006).
12. A. A. Disney, C. Aoki, M. J. Hawken, *Neuron* **56**, 701 (2007).
13. G. T. Prusky, C. Shaw, M. S. Cynader, *Brain Res.* **412**, 131 (1987).
14. D. Parkinson, K. E. Kratz, N. W. Daw, *Exp. Brain Res.* **73**, 553 (1988).
15. Z. Gil, B. W. Connors, Y. Amitai, *Neuron* **19**, 679 (1997).
16. I. Kruglikov, B. Rudy, *Neuron* **58**, 911 (2008).
17. E. Lucas-Meunier et al., *Cereb. Cortex* **19**, 2411 (2009).
18. M. C. Kuo, D. D. Rasmuson, H. C. Dringenberg, *Neuroscience* **163**, 430 (2009).
19. P. Aracri et al., *Cereb. Cortex* **20**, 1539 (2010).
20. M. Alkondon, E. F. R. Pereira, H. M. Eisenberg, E. X. Albuquerque, *J. Neurosci.* **20**, 66 (2000).
21. E. O. Mann, I. Mody, *Curr. Opin. Neurol.* **21**, 155 (2008).
22. R. Satta et al., *Proc. Natl. Acad. Sci. U.S.A.* **105**, 16356 (2008).
23. Q. Gu, W. Singer, *Eur. J. Neurosci.* **5**, 475 (1993).
24. J. L. Herrero et al., *Nature* **454**, 1110 (2008).
25. M. Goard, Y. Dan, *Nat. Neurosci.* **12**, 1444 (2009).
26. J. I. Kang, E. Vaucher, *PLoS ONE* **4**, e5995 (2009).
27. D. M. Levi, R. W. Li, *Philos. Trans. R. Soc. Lond. B Biol. Sci.* **364**, 399 (2009).
28. M. W. Dye, C. S. Green, D. Bavelier, *Neuropsychologia* **47**, 1780 (2009).
29. M. A. Silver, A. Shenav, M. D'Esposito, *Neuron* **60**, 904 (2008).
30. R. F. Hess, B. Thompson, G. Gole, K. T. Mullen, *Eur. J. Neurosci.* **29**, 1064 (2009).
31. P. Lempert, *Ophthalmic Physiol. Opt.* **25**, 592 (2005).
32. M. Sarter, M. E. Hasselmo, J. P. Bruno, B. Givens, *Brain Res. Brain Res. Rev.* **48**, 98 (2005).
33. M. F. Bear, W. Singer, *Nature* **320**, 172 (1986).
34. C. K. Pfeffer et al., *J. Neurosci.* **29**, 3419 (2009).
35. J. F. Mitchell, K. A. Sundberg, J. H. Reynolds, *Neuron* **55**, 131 (2007).
36. Y. Chen et al., *Nat. Neurosci.* **11**, 974 (2008).
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Motor Control by Sensory Cortex

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Classical studies of mammalian movement control define a prominent role for the primary motor cortex. Investigating the mouse whisker system, we found an additional and equally direct pathway for cortical motor control driven by the primary somatosensory cortex. Whereas activity in primary motor cortex directly evokes exploratory whisker protraction, primary somatosensory cortex directly drives whisker retraction, providing a rapid negative feedback signal for sensorimotor integration. Motor control by sensory cortex suggests the need to reevaluate the functional organization of cortical maps.

The remarkable findings of Penfield and Boldrey (1), which have been supported by many subsequent studies (2–11), emphasize a key role for motor cortex in mammalian movement control. Investigating the mouse whisker system (12–14), we found that primary somato-

sensory barrel cortex forms an equally direct and equally prominent motor control pathway, compared with that originating from the classical motor cortex.

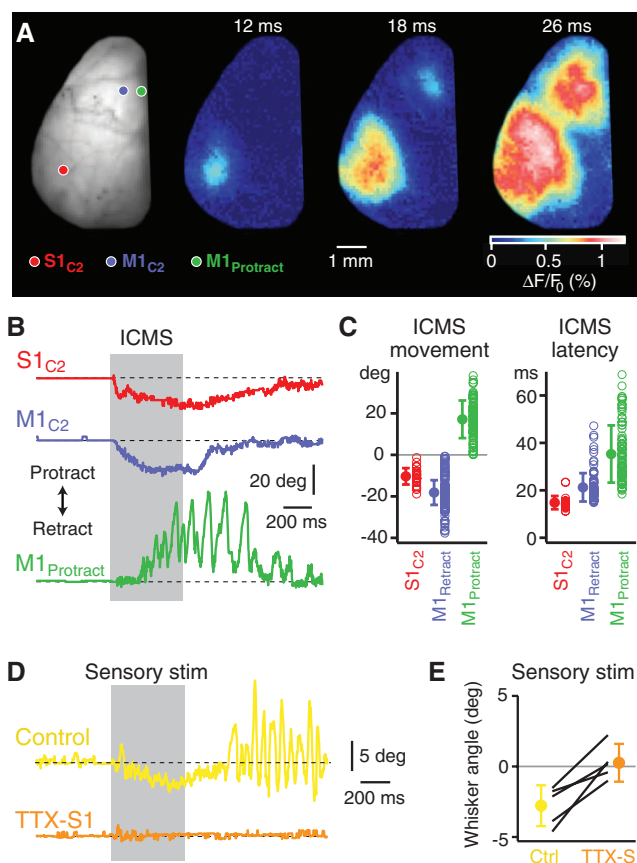
We first functionally mapped the sensory activity evoked by a single brief deflection of the C2 whisker through wide-field voltage-sensitive dye

(VSD) imaging of the contralateral sensorimotor cortex in awake head-restrained mice (15, 16). The earliest cortical VSD response to C2 whisker deflection occurred at 7.4 ± 0.5 ms ($n = 5$ mice, mean $\pm$ SD) and was specifically localized to the C2 barrel column of primary somatosensory neocortex (S1_{C2}) (Fig. 1A). Over the subsequent milliseconds, nearby cortical columns depolarized, with activity propagating in a wavelike manner.

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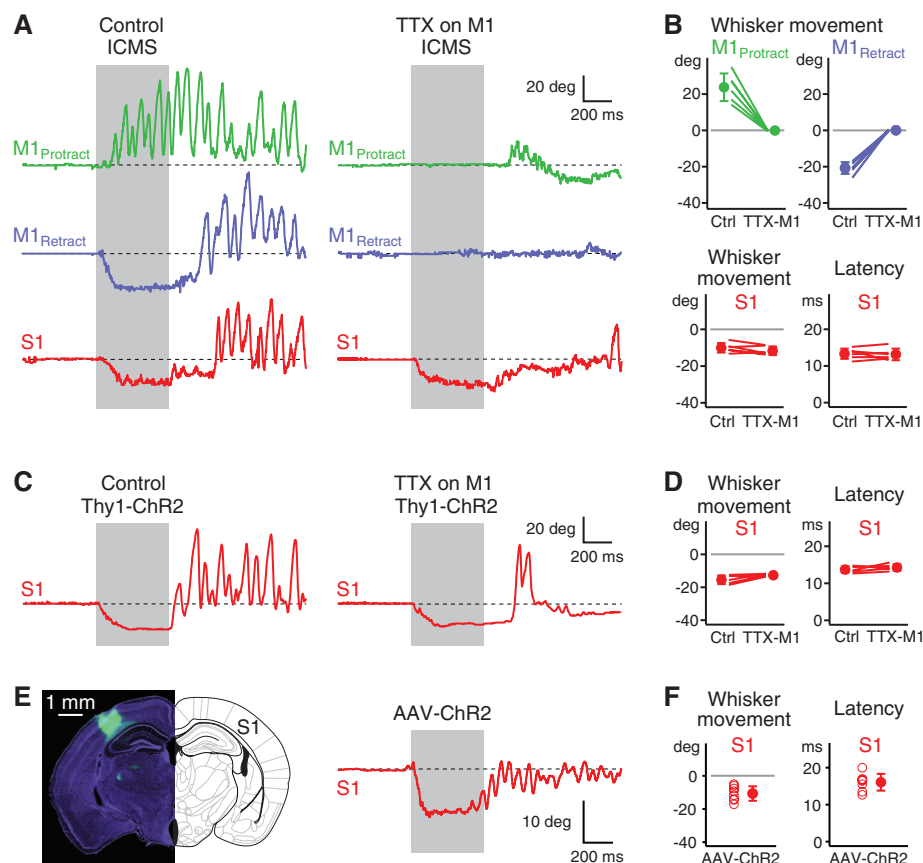
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Fig. 1. Cortical sensorimotor interactions in the mouse whisker system. (A) VSD imaging reveals the spatio-temporal dynamics of the cortical sensory response evoked by a single brief deflection of the C2 whisker. (B) ICMS (gray shading) of S1_{C2} (red) and M1_{C2} (blue) evoked whisker retraction, whereas ICMS of M1_{Protract} (green) drove whisker protraction. (C) Movement amplitude and latency evoked by ICMS of S1_{C2} ($n = 26$ mice), M1_{Retract} (including M1_{C2}, $n = 31$ mice), and M1_{Protract} ($n = 30$ mice). (D and E) Sensory-evoked whisker retraction (yellow) was blocked by TTX inactivation of S1 (orange) ($P < 0.01$, $n = 5$ mice). Data presented as mean $\pm$ SD.



At 6.5 ± 1.9 ms ($n = 5$ mice) after the first excitation of the C2 barrel column, a second localized region of depolarization was evoked in primary motor cortex (M1_{C2}), which also spread over the next milliseconds (Fig. 1A). These imaging experiments defined two spatially localized initiation sites (S1_{C2} and M1_{C2}) for cortical sensory processing associated with the C2 whisker. To gain insight into their functional contributions to whisker behavior, we targeted intracortical microstimulation (ICMS) to these two cortical regions, finding that stimulation of either S1_{C2} or M1_{C2} evoked short-latency retraction of the C2 whisker ($n = 5$ mice) (Fig. 1B). Stimulation of a more medial region of motor cortex (M1_{Protract}) drove rhythmic whisker protraction (Fig. 1B). Additional mapping experiments based on stereotaxic coordinates revealed this motor map to be well defined, with M1_{C2} localized within the motor cortex whisker retraction area, M1_{Retract} (figs. S1 and S2) (8, 15). Quantified across all experiments (Fig. 1C), ICMS of S1_{C2} drove whisker retraction of $-10.3^\circ \pm 3.9^\circ$ with a latency of 14.8 ± 2.8 ms ($n = 26$, stimulation sites across 26 mice); ICMS of M1_{Retract} drove whisker retraction of $-18.0^\circ \pm 8.8^\circ$ with a latency of 21.1 ± 5.8 ms ($n = 116$, across 31 mice); and ICMS of M1_{Protract} drove whisker protraction of $17.1^\circ \pm 9.0^\circ$ with a latency of 35.3 ± 12.1 ms ($n = 86$, across 30 mice). Latencies for evoking whisker movements were shorter for ICMS of S1 compared with those of M1 ($P < 0.001$).

Fig. 2. Sensory cortex drives whisker retraction independently of motor cortex. (A and B) Inactivation of M1 by TTX, completely blocked movements evoked by intracortical microstimulation (ICMS) of M1_{Protract} (green) and M1_{Retract} (blue) but did not affect whisker retraction driven by ICMS of S1 (red) in the same mice ($n = 7$ mice). Note that spontaneous whisker movement can still occur following inactivation of M1 by TTX (see M1_{Protract} trace). (C and D) Optogenetic stimulation of S1 in ChR2 transgenic mice evoked whisker retraction, which was unchanged by inactivation of M1 by TTX ($n = 5$ mice). (E and F) Whisker retraction evoked by optogenetic stimulation of S1 neurons expressing ChR2 from an adeno-associated viral vector ($n = 8$ mice). Data presented as mean $\pm$ SD.



Thus, the cortical regions involved in the initial processing of C2 whisker sensory input (S1_{C2} and M1_{C2}) both drove whisker retraction. To test for behavioral relevance of the retraction motor response from sensory cortex, we attached metal particles to the C2 whisker and evoked a train of brief whisker deflections by a pulsed magnetic field. Mice retracted the C2 whisker in response to this sensory stimulus by $-2.8^\circ \pm 1.4^\circ$ ($n = 5$ mice), but, if the S1 barrel cortex was inactivated by tetrodotoxin (TTX), then the mice failed to respond (whisker movement of $0.3^\circ \pm 1.2^\circ$, $n = 5$ mice, $P < 0.01$) (Fig. 1, D and E). Inactivation of motor cortex by TTX did not affect the sensory-evoked whisker retraction (fig. S3).

These results suggest that S1 might drive movement without the participation of M1. We tested this hypothesis by investigating the effect of TTX application to M1 on ICMS-evoked movements. Although complete blockade of motor cortex was verified by the absence of movements evoked by ICMS of M1, the whisker retraction evoked by ICMS of S1 was unaffected by TTX application to M1 (control amplitude of $-11.0^\circ \pm 3.4^\circ$, TTX_{M1} amplitude of $-11.6^\circ \pm 2.1^\circ$; control latency 13.0 ± 1.5 ms, TTX_{M1} latency 12.9 ± 1.6 ms; $n = 7$ mice) (Fig. 2, A and B).

ICMS evokes widespread activity under some experimental conditions (15, 17, 18). We therefore tested for S1-evoked movements by using a second independent method that involved optogenetic stimulation of neurons expressing ChR2 (19, 20). In Thy1-ChR2 transgenic mice (21, 22), robust whisker retraction could be evoked by blue light stimulation of S1 barrel cortex (Fig. 2C). Application of TTX to motor cortex completely blocked movements evoked by optogenetic stimulation of motor cortex, but it had no effect upon S1-evoked optogenetic whisker retraction (S1 control amplitude $-15.5^\circ \pm 2.7^\circ$, S1 TTX_{M1} amplitude $-12.7^\circ \pm 0.9^\circ$; S1 control latency 13.7 ± 0.9 ms, S1 TTX_{M1} latency 14.3 ± 0.9 ms; $n = 5$ mice) (Fig. 2, C and D, and movie S1). To achieve the highest specificity for optogenetic stimulation, we used an adeno-associated viral (AAV) vector to locally express ChR2 in S1 barrel cortex (Fig. 2E). Blue light stimulation of these S1 neurons drove a robust short-latency whisker retraction in 8 out of 11 mice (amplitude $-10.8^\circ \pm 3.2^\circ$, latency 16.1 ± 1.8 ms, $n = 8$ mice) (Fig. 2, E and F).

We next began to question whether the movements evoked by stimulation of M1 were driven directly by motor cortex or whether the evoked whisker retraction might actually be relayed via S1. Whisker protraction driven by ICMS of M1_{Protract} was enhanced by TTX inactivation of S1 ($n = 13$ mice) (Fig. 3, A and B). However, the whisker retraction evoked under control conditions by ICMS of M1_{Retract} was reversed after TTX application to S1 and was replaced by rhythmic protraction (control amplitude $-18.0^\circ \pm 5.6^\circ$, TTX_{S1} amplitude $21.6^\circ \pm 9.9^\circ$, $n = 14$ mice) (Fig. 3, A and B). Similarly, the retraction evoked by optogenetic stimulation of M1_{Retract} in Thy1-ChR2 mice was also reversed into protraction by TTX application to S1 ($n = 5$ mice) (Fig. 3, C and D, and movie S2). In a separate set of

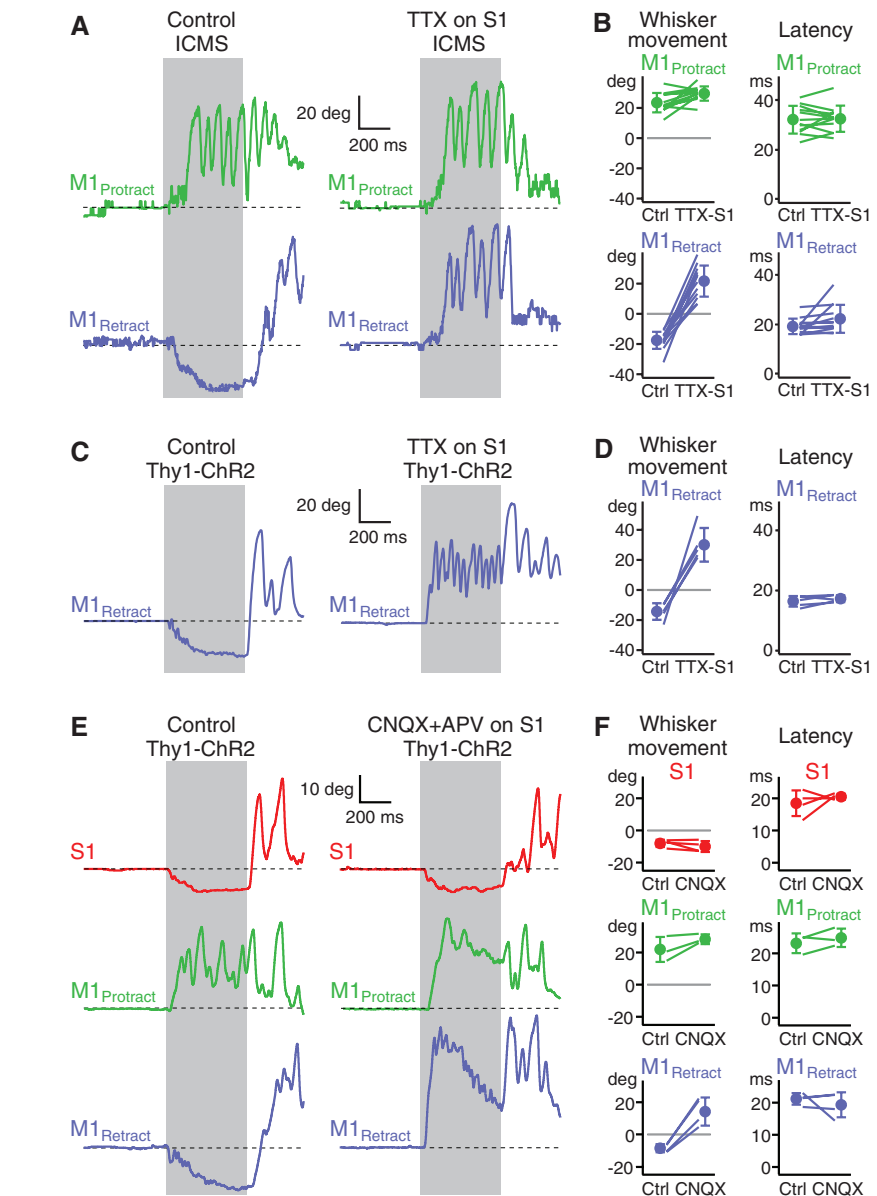


Fig. 3. Whisker retraction evoked by stimulation of motor cortex is driven by sensory cortex. (A and B) Inactivation of S1 enhanced whisker protraction evoked by ICMS of M1_{Protract} (green, $n = 13$ mice, $P < 0.01$), but in the same mice it reversed the movements evoked by ICMS of M1_{Retract} (blue, $n = 14$ mice, $P < 0.001$) from retraction into protraction. (C and D) Whisker retraction evoked by optogenetic stimulation of M1_{Retract} in Thy1-ChR2 transgenic mice was also changed into whisker protraction after S1 inactivation by TTX ($P < 0.01$, $n = 5$ mice). (E and F) Application of CNQX and APV to S1 did not affect whisker movements evoked by optogenetic stimulation of S1 (red; $n = 4$ mice) or M1_{Protract} (green; $n = 3$ mice), but whisker retraction driven by optogenetic stimulation of M1_{Retract} was changed into protraction (blue; $P < 0.01$, $n = 4$ mice). Data presented as mean $\pm$ SD.

experiments, applying 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX) and D-2-amino-5-phosphonopentanoic acid (APV) to S1 [to block AMPA and N-methyl-D-aspartate (NMDA) types of ionotropic glutamate receptors, respectively], we determined that the whisker retraction evoked by optogenetic stimulation of M1_{Retract} is mediated by a glutamatergic synapse in S1, whereas neither the protraction evoked by stimulation of M1_{Protract} nor the retraction evoked by stimulation of S1 required glutamatergic synaptic transmission in S1 (Fig. 3, E and F). These results reveal that the direct action of whisker primary

motor cortex neurons (both M1_{Retract} and M1_{Protract}) is to drive whisker protraction. The whisker retraction evoked by stimulation of motor cortex area M1_{Retract} is in fact driven indirectly, but reliably, via synaptic activation of S1.

Having defined different functional motor roles for M1 (whisker protraction) and S1 (whisker retraction), we next investigated the downstream signaling pathways. Consistent with a major feed-forward pathway for sensory information from S1 to M1 visualized with VSD (Fig. 1), we found a high-density column of axons from S1_{C2} in the

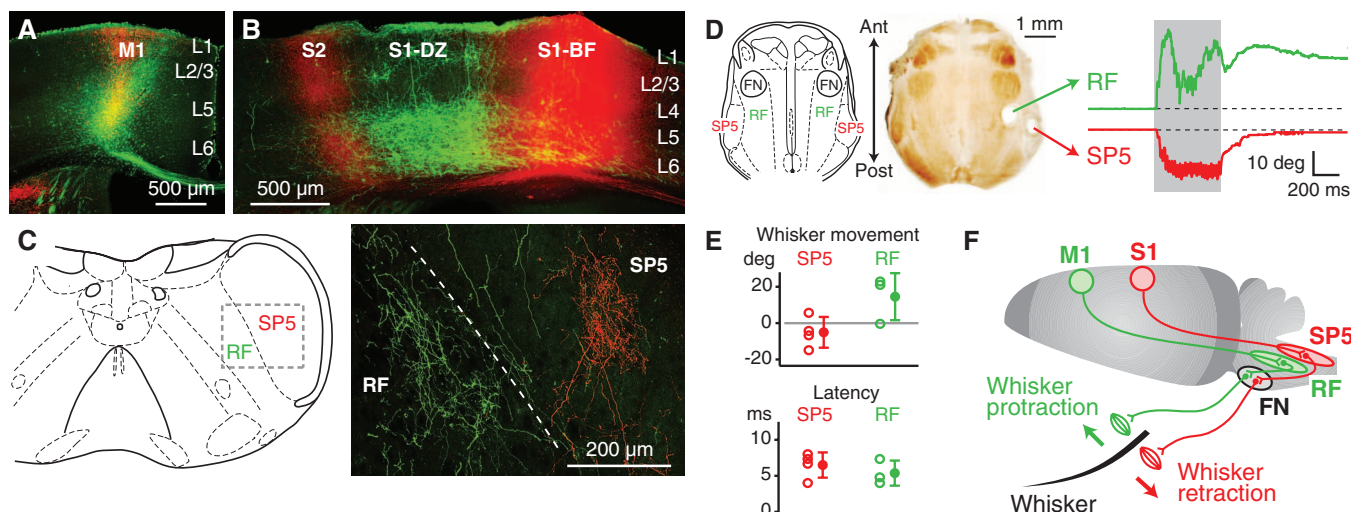


Fig. 4. Motor signaling pathways from S1 and M1. (A) Lentivirus-labeled axonal projection into M1 by neurons located in the C2 barrel column of S1 (red). Biotinylated dextran amine (BDA) was injected into this region of M1 (green). In the overlay, colocalization appears in yellow. (B) The lentiviral injection site in S1 barrel field (S1-BF) of the same mouse is strongly labeled along with the axonal projection to S2 (red). The BDA-labeled axonal projections into S1 from M1 (green) arborize primarily in layers 1, 5, and 6 of the barrel field (S1-BF) and the surrounding dysgranular zone (S1-DZ). (C) M1 (green) projects to the brainstem reticular

formation (RF), and S1 (red) projects to spinal trigeminal nuclei (SP5) of the same mouse. Schematic drawing (left) adapted from Paxinos and Franklin (33). (D) Microstimulation of RF evoked whisker protraction, whereas microstimulation of SP5 drove whisker retraction. Microstimulation locations were marked with lesions, and fixed sections were cytochrome oxidase stained. (E) Amplitudes and latencies for whisker movement evoked by microstimulation of SP5 ($n = 4$ mice) and RF ($n = 3$ mice) (mean $\pm$ SD). (F) Schematic drawing of two parallel whisker motor pathways from the cortex to the motor neurons located in the facial nucleus (FN).

location of M1_{C2} (Fig. 4A) (15, 23). The reciprocal axonal projection from M1_{C2} preferentially innervated L1 and L5/6 of the S1 barrel field (S1-BF) and the neighboring dysgranular zone (S1-DZ) (Fig. 4B) (24). Whereas the corticocortical projections of S1_{C2} and M1_{C2} were quite different from each other, the overall map of subcortical projections followed a similar pattern. Both S1_{C2} and M1_{C2} strongly innervated adjacent (but mainly nonoverlapping) brain regions involved in processing whisker sensation and movement, that is, dorsal striatum, thalamus, superior colliculus, red nucleus, pontine nuclei, and trigeminal brainstem (Fig. 4C and fig. S4).

Whisker movements evoked by motor cortex stimulation are likely mediated by the prominent M1_{C2} innervation of the brainstem reticular formation (RF) (Fig. 4C), which projects heavily into the facial nucleus (FN), where the whisker motor neurons are located (25, 26). In an analogous parallel pathway, the most prominent brainstem projection of S1_{C2} is to the spinal trigeminal nuclei (SP5) (Fig. 4C) (23, 27), which project strongly to the facial nucleus (25, 28). Direct electrical stimulation of SP5 evoked whisker retraction (amplitude $-5.1^\circ \pm 8.5^\circ$, latency 6.5 ± 1.8 ms, $n = 4$ mice), whereas stimulation of RF drove whisker protraction (amplitude $14.4^\circ \pm 12.9^\circ$, latency 5.4 ± 1.7 ms, $n = 3$ mice) (Fig. 4, D and E). We thus propose the existence of two parallel motor signaling pathways, which emerge from distinct cortical areas (M1 and S1) and are relayed via distinct nuclei in the brainstem (RF and SP5) to antagonistic pools of motor neurons in FN driving different whisker-related muscles (29): M1 drives whisker protraction (M1 $\rightarrow$ RF $\rightarrow$ FN), and S1 drives whisker retraction (S1 $\rightarrow$ SP5 $\rightarrow$ FN) (Fig. 4F).

We found evidence for a strong and direct role for mouse sensory cortex in whisker motor control.

Given the importance of sensorimotor interactions during any form of active sensing, one should next examine whether reliable and direct motor control by sensory cortex is a general feature or whether it is a specialization of the mouse whisker sensorimotor system. Interestingly, previous investigations found overlapping sensory and motor representations of the rodent hindlimb (2). Furthermore, in the monkey, corticospinal neurons are present in S1 (30), and retrograde trans-synaptic tracing from finger muscles labels neurons in S1 (31). In this context, it is also interesting to note that early motor mapping studies in monkeys (32) and humans (1) describe movements evoked by stimulation of primary somatosensory cortex in addition to motor cortex. Motor control by sensory cortex, as demonstrated by our experiments in the mouse whisker system, might therefore also be relevant to the functional organization of human cortex.

References and Notes

- W. Penfield, E. Boldrey, *Brain* **60**, 389 (1937).
- J. P. Donoghue, S. P. Wise, *J. Comp. Neurol.* **212**, 76 (1982).
- A. P. Georgopoulos, A. B. Schwartz, R. E. Kettner, *Science* **233**, 1416 (1986).
- J. Wessberg et al., *Nature* **408**, 361 (2000).
- M. D. Serruya, N. G. Hatsopoulos, L. Paninski, M. R. Fellows, J. P. Donoghue, *Nature* **416**, 141 (2002).
- M. S. Graziano, C. S. Taylor, T. Moore, *Neuron* **34**, 841 (2002).
- M. Brecht, M. Schneider, B. Sakmann, T. W. Margrie, *Nature* **427**, 704 (2004).
- F. Haiss, C. Schwarz, *J. Neurosci.* **25**, 1579 (2005).
- D. A. Dombeck, M. S. Graziano, D. W. Tank, *J. Neurosci.* **29**, 13751 (2009).
- Y. Isomura, R. Harukuni, T. Takekawa, H. Aizawa, T. Fukai, *Nat. Neurosci.* **12**, 1586 (2009).
- T. Komiyama et al., *Nature* **464**, 1182 (2010).
- M. Brecht, *Curr. Opin. Neurobiol.* **17**, 408 (2007).
- C. C. H. Petersen, *Neuron* **56**, 339 (2007).
- M. E. Diamond, M. von Heimendahl, P. M. Knutsen, D. Kleinfeld, E. Ahissar, *Nat. Rev. Neurosci.* **9**, 601 (2008).
- I. Ferezou et al., *Neuron* **56**, 907 (2007).
- Materials and methods are available as supporting material on Science Online.
- E. Seidemann, A. Arieli, A. Grinvald, H. Slovin, *Science* **295**, 862 (2002).
- M. H. Histed, V. Bonin, R. C. Reid, *Neuron* **63**, 508 (2009).
- G. Nagel et al., *Proc. Natl. Acad. Sci. U.S.A.* **100**, 13940 (2003).
- E. S. Boyden, F. Zhang, E. Bamberg, G. Nagel, K. Deisseroth, *Nat. Neurosci.* **8**, 1263 (2005).
- B. R. Arenkiel et al., *Neuron* **54**, 205 (2007).
- O. G. Ayling, T. C. Harrison, J. D. Boyd, A. Goroshkov, T. H. Murphy, *Nat. Methods* **6**, 219 (2009).
- R. Aronoff et al., *Eur. J. Neurosci.* **31**, 2221 (2010).
- P. Veinante, M. Deschênes, *J. Comp. Neurol.* **464**, 98 (2003).
- A. M. Hattox, C. A. Priest, A. Keller, *J. Comp. Neurol.* **442**, 266 (2002).
- L. J. Herfst, M. Brecht, *J. Neurophysiol.* **99**, 2821 (2008).
- M. F. Jacquin, M. R. Wiegand, W. E. Reinehan, *J. Neurophysiol.* **64**, 3 (1990).
- G. Pingaud, I. Bernat, P. Buisseret, C. Buisseret-Delmas, *J. Comp. Neurol.* **415**, 91 (1999).
- D. N. Hill, R. Bermejo, H. P. Zeigler, D. Kleinfeld, *J. Neurosci.* **28**, 3438 (2008).
- J. D. Coulter, E. G. Jones, *Brain Res.* **129**, 335 (1977).
- J. A. Rathelot, P. L. Strick, *Proc. Natl. Acad. Sci. U.S.A.* **103**, 8257 (2006).
- W. I. Welker, R. M. Benjamin, R. C. Miles, C. N. Woolsey, *J. Neurophysiol.* **20**, 347 (1957).
- G. Paxinos, K. Franklin, *The Mouse Brain in Stereotaxic Coordinates* (Academic Press, San Diego, CA, ed. 2, 2001).
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Supporting Online Material

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Materials and Methods

Figs. S1 to S4

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Movies S1 and S2

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Poly(A) Tail Recognition by a Viral RNA Element Through Assembly of a Triple Helix

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Kaposi's sarcoma–associated herpesvirus produces a highly abundant, nuclear noncoding RNA, polyadenylated nuclear (PAN) RNA, which contains an element that prevents its decay. The 79-nucleotide expression and nuclear retention element (ENE) was proposed to adopt a secondary structure like that of a box H/ACA small nucleolar RNA (snoRNA), with a U-rich internal loop that hybridizes to and protects the PAN RNA poly(A) tail. The crystal structure of a complex between the 40-nucleotide ENE core and oligo(A)₉ RNA at 2.5 angstrom resolution reveals that unlike snoRNAs, the U-rich loop of the ENE engages its target through formation of a major-groove triple helix. A-minor interactions extend the binding interface. Deadenylation assays confirm the functional importance of the triple helix. Thus, the ENE acts as an intramolecular RNA clamp, sequestering the PAN poly(A) tail and preventing the initiation of RNA decay.

Kaposi's sarcoma–associated herpesvirus (KSHV) is the causative agent of Kaposi's sarcoma (KS), the most common AIDS-associated cancer (1, 2). Although largely controlled in the developed world by antiretroviral treatment against HIV, KS has become one of the

most prevalent cancers in Africa (3). KSHV is a γ -herpesvirus that exists in either a latent or lytic state. During the lytic phase, KSHV produces PAN (polyadenylated nuclear) RNA, a 1.1-kb noncoding RNA with a 5' cap and 3' poly(A) tail (4–6) that is retained in the nucleus of infected cells. Although the function of PAN RNA is not known, it accumulates to extremely high levels, amounting to as much as 80% of the polyadenylated RNA in the cell (4, 6). The expression and nuclear retention element (ENE), a 79-nucleotide (nt) element located near the 3' end of PAN RNA, is responsible for this accumulation (7). The ENE prevents deadenylation and decay of PAN RNA through direct, cis-acting sequestration of the poly(A) tail (8). The ENE also abrogates rapid nuclear decay when inserted into polyadenylated mRNAs lacking introns (8), apparently through protection

from deadenylation, the first step in degradation of eukaryotic mRNAs (9, 10). Secondary structure prediction and analyses of mutants using cellular assays suggested that the ENE forms a hairpin containing a U-rich internal loop flanked by short helices, reminiscent of box H/ACA small nucleolar RNA (snoRNA) hairpins (Fig. 1A) (8, 11). The internal loops of box H/ACA snoRNAs base pair with target regions in ribosomal RNA (rRNA) to direct the conversion of specific uridine residues to pseudouridine (12). By analogy, the ENE internal loop was predicted to base pair with the poly(A) tail of PAN RNA (Fig. 1A), protecting it from exonucleolytic digestion (11).

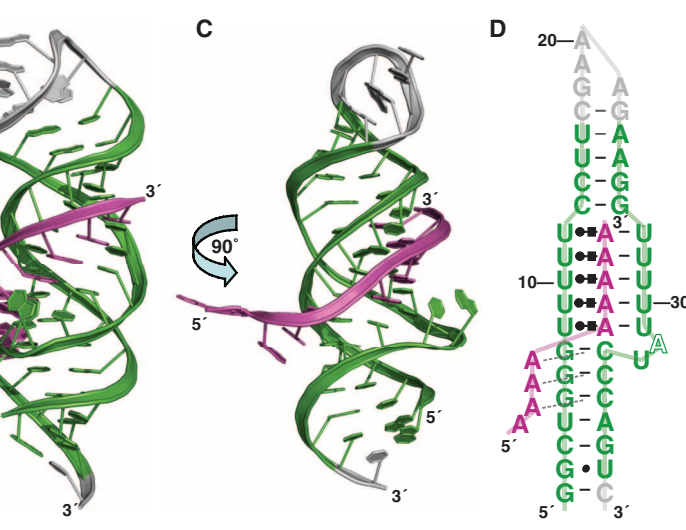
To elucidate the mode of interaction between the ENE and the poly(A) tail of PAN RNA, we have determined the crystal structure of a complex formed between the 40-nt ENE core and oligo(A) RNA and studied it biochemically (13). Electrophoretic mobility shift assays showed that the isolated ENE is capable of binding oligo(A) in trans (fig. S1). U to C mutations in the internal loop greatly decrease the interaction between the ENE and oligo(A), whereas truncations of the ENE that retain the helix-loop-helix core (Fig. 1A) have no effect (fig. S1). These results are consistent with those obtained from in vivo and in vitro assays of ENE mutants (11). Nuclear magnetic resonance (NMR) studies confirmed that the ENE core alone adopts a helix-loop-helix conformation (fig. S2A). Increased spectral complexity in the imino region of one-dimensional ¹H spectra upon A₁₀ addition suggested that the loop becomes involved in hydrogen-bonding interactions with the oligo(A) (fig. S2B). Crystallization trials were then performed using several ENE core sequences that varied in the helix length mixed with a variety of short A-rich RNA oligonucleotides. Optimal crystallization occurred with the 40-

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Fig. 1. Structural overview of the complex formed between the ENE core and A₉ RNA. **(A)** Schematic diagram of PAN RNA, showing the interaction between the ENE and the poly(A) tail that was previously proposed (8, 11), with the ENE core in green. For crystallization, the upper stem of this central sequence was capped with a C-G base pair and a GAAA tetraloop and the lower stem was followed by a 3' C nucleotide (to produce a blunt-ended helix) (Fig. 1D). **(B)** Ribbon representation of the crystal structure, with the ENE core in green, the non-native sequence in gray, and the A₉ oligonucleotide in magenta. Four copies of the ENE complex are found in the asymmetric unit of the crystal, two pairs of complexes related by noncrystallographic symmetry (NCS). The two non-NCS-related complexes are almost identical in the central region [RMSD = 0.57 Å over all nonhydrogen



atoms in the triple helix (fig. S3)]. **(C)** 90° rotation of the image shown in (B). **(D)** Schematic diagram of the complex [colors as in (B and C)], with Leontis-Westhof notation (26) indicating major-groove triple helix contacts and dotted lines indicating A-minor interactions. The base of the bulged nucleotide A32 (outlined) is disordered.

nt ENE core construct and A₉ RNA. Data from crystals soaked in buffer solutions containing iridium hexamine were used to determine the phases, and the final structure was refined to 2.5 Å resolution ($\langle I/\sigma \rangle = 1.0$; $\langle I/\sigma \rangle = 2.0$ at 2.65 Å resolution) with working and free *R* factors (R_{work} and R_{free}) of 21.8%/23.3%, respectively (figs. S3 to S5 and table S1).

The crystal structure reveals that the ENE core forms a triple-stranded complex with its bound A₉ oligonucleotide (Fig. 1). The ENE assumes

the expected secondary structure, with Watson-Crick stems flanking a U-rich internal loop (10). Instead of forming base pairs around this loop, however, the A₉ RNA adopts an extended conformation and interacts with both the ENE loop and the lower stem. Nucleotides A5 to A9 of the A₉ oligonucleotide simultaneously engage both sides of the internal loop in an extended U-A•U major-groove triple helix (Fig. 2A). Here, the five consecutive A nucleotides form Watson-Crick base pairs with the five consecutive U nucleo-

tides on the 3' side of the loop [U27 to U31 (14)]. The 5' side of the ENE internal loop lies in the major groove of the resulting helix, with the Watson-Crick face of nucleotides U8 to U12 base pairing with the Hoogsteen face of the A nucleotides (Fig. 2B). Hydrogen bonds are also observed between the A₉ phosphate backbone and the ribose hydroxyl groups of the Hoogsteen U strand. The base triples formed are nearly planar, and there is no direct contact between the two U strands of the internal loop (Fig. 2, A and B). The

Fig. 2. Detailed views of key structural interactions between the ENE and A₉ RNA. (A) Close-up of the major-groove triple helix formed by 5 nt of A₉ and the internal U-rich loop of the ENE hairpin (colors as in Fig. 1, with the bases involved in Watson-Crick pairing in yellow, hydrogen bonds within base triples in cyan, and the A₉ nucleotides labeled in italics). The U strand that makes Hoogsteen interactions in the major groove is shown in the foreground. (B) Superposition of the five ENE:A₉ U-A•U base triples showing the regularity of the triple-helical structure. (C) Close-up of the A-minor triad, with Watson-Crick base pairing and hydrogen bonds involving the A strand in cyan. (D) A-minor interactions with the lower ENE stem. Crystal-packing interactions involving nucleotide A1 (fig. S3A) most likely pull the N2 of nucleotide A2 just beyond hydrogen-bonding distance from the 2' OH of C36 in the Type III interaction.

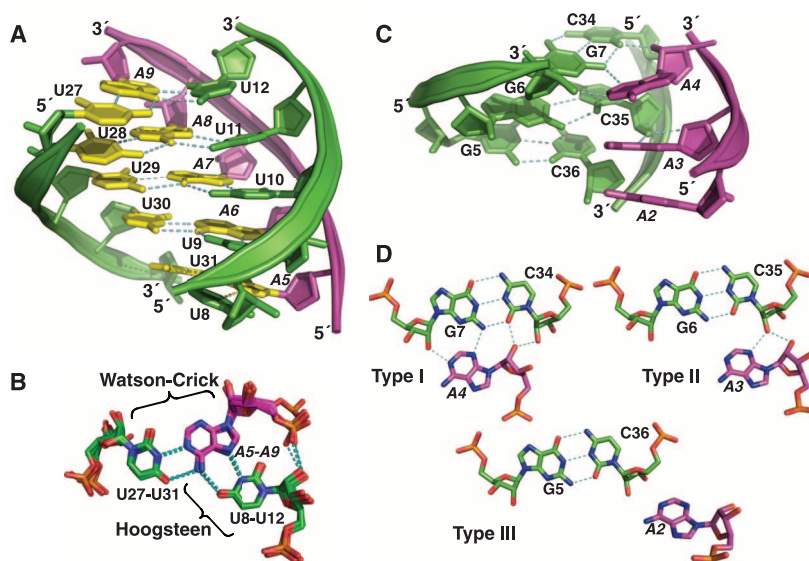
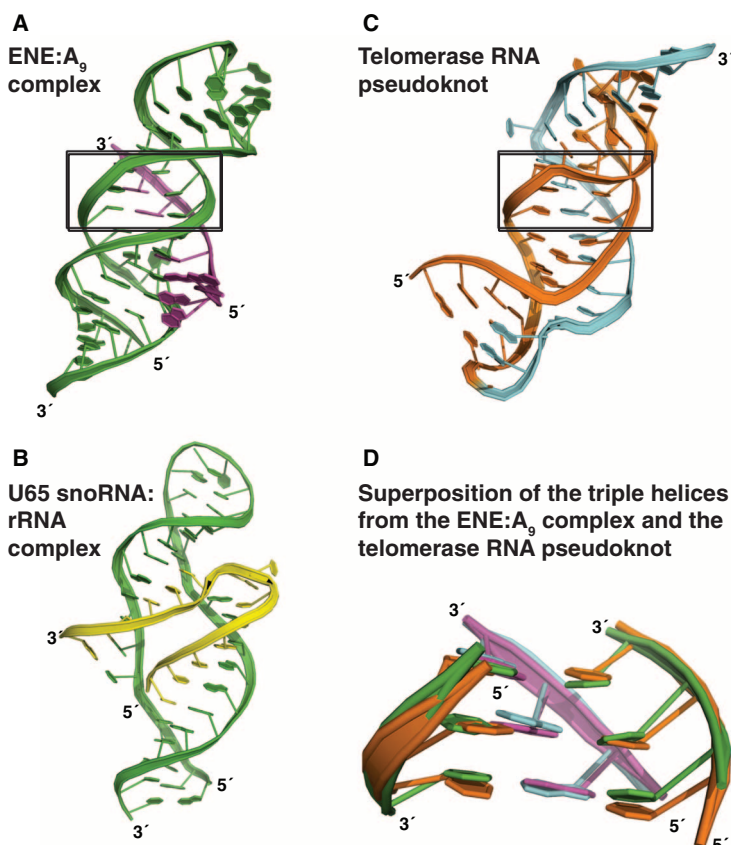


Fig. 3. Comparison of the ENE:A₉ complex with other structures. (A) The complex between the ENE and A₉ RNA (top three base triples boxed). (B) The solution structure of a complex between a truncated H/ACA snoRNA and a 14-nt sequence corresponding to its rRNA substrate [PDB 2P89, model 1 (18)]. The snoRNA hairpin is shown in green, with the bound substrate in yellow. (C) The solution structure of the human telomerase pseudoknot [PDB 2K95, model 1 (27)]. The triple helix (boxed) is composed of three U-A•U base triples, and the strand contributing these A nucleotides is shown in cyan, whereas the strands that contribute the U nucleotides are shown in brown. (D) Superposition of the triple helix from the ENE:A₉ complex with the triple helix from the telomerase RNA pseudoknot. Colors and orientations are as in (A) and (C). The top three U-A•U base triples (involving A7 to A9) from the ENE:A₉ complex were used for superposition (RMSD = 1.1 Å over all nonhydrogen atoms).



helical axis of the complex is nearly straight through the transitions between the triple helix and the flanking ENE stems. The additional two nucleotides of the 3' side of the ENE internal loop (A32 and U33) bulge out, allowing continuous stacking between the lower stem and the A and Hoogsteen strands of the triple helix. Despite the presence of the Hoogsteen strand in the A:U major groove, a deep groove remains (fig S6).

The binding interface is augmented by a triad of A-minor interactions formed between the bound A₉ oligonucleotide and the lower stem of the ENE. Nucleotides A2 to A4 contact the three consecutive G-C base pairs that close the lower stem (Fig. 2C). As previously described for 23S rRNA in the high-resolution structure of the 50S ribosome (15), the three As of the triad penetrate with increasing depth into the G:C minor groove from the 5' to 3' direction, such that the initial interaction is a type III, the next is a type II, and the last is a type I A-minor interaction (Fig. 2D) (15, 16).

The interactions made between the ENE core and A₉ RNA are strikingly different from those determined for the box H/ACA snoRNAs and their target rRNA sequences (Fig. 3, A and B). In the snoRNA structures, the substrate strand is sharply kinked, forming parallel Watson-Crick helices with the two strands of the snoRNA internal loop, which then stack coaxially on the flanking snoRNA hairpin helices (17, 18). The crystal structure described here involves an intermolecular interaction between the core ENE and A₉ RNA. However, as the ENE has only been observed to protect the PAN RNA poly(A) tail in an intramolecular manner (8), these elements formally adopt a pseudoknot structure in the context of the full-length RNA (see supporting online material). Consistent with this, the ENE:A₉ complex resembles H-type pseudoknots observed in telomerase RNA (Fig. 3, C and D) (19) and the S-adenosylmethionine-responsive riboswitch SAM-II (fig. S7) (20). Although the topology of the surrounding stems is different in the H-type pseudoknots, the structures of the major-groove triple helices can be superimposed with that of the ENE complex with root mean square deviations (RMSDs) of ~1 Å. Felsenfeld, Davies, and Rich first studied RNA triple helices composed of poly(U)-poly(A)•poly(U) more than half a century ago (21, 22). Denaturation studies later suggested that DNA hairpins with T-rich loops likewise bind oligo(dA) through formation of T-A•T triple helices (23). Analogous interactions between RNA hairpins and A-rich sequences were also proposed (23). However, the ENE is the first natural example of the use of a U-rich internal loop to capture and sequester a poly(A) RNA sequence.

We used deadenylation assays to confirm the functional importance of the ENE:A₉ triple helix described here (13). Mutation of a single U to C in either side of the ENE U-rich pocket had previously been shown to decrease protection of PAN RNA's poly(A) tail from deadenylation in nuclear extract (11). Simultaneous U to C mutations (mutating one nucleotide from each side of the pocket) were as deleterious as deletion of the entire ENE.

Because the two U nucleotides mutated in that experiment contact the same A in the crystal structure (Fig. 4A), we postulated that the loss of ENE function arising from disruption of a single U-A•U base triple might be restored by replacement with a nearly isosteric C-G•C base triple (Fig. 4B) (24). Native gel shift analysis supported this proposal, as ENEs containing simultaneous U to C mutations contacting the same A in the crystal structure can bind an oligo(A) molecule containing a single A to G substitution (A₇GA₂) (fig. S8, A and C), whereas ENE constructs with mutations to C in U residues that contact two different A nucleotides in the crystal structure cannot (fig. S8, B and C).

We thus transcribed PAN RNA deadenylation substrates that contained either wild-type or double-mutant ENE or that lacked the ENE altogether (see legend to Fig. 4C). Each of the substrates terminated in a 60-nt poly(A) tail with or without a single A to G substitution (A₆₀ or A₅₇GA₂). We assessed these constructs for ENE-dependent protection from deadenylation in nuclear extract (Fig. 4C, left and middle panels). As expected, the wild-type ENE protected the A₆₀ tail from de-

adenylation, whereas the A₆₀ tails of constructs containing no ENE (Δ ENE) or the double-mutant ENE (U903C, U949C) were not protected from deadenylation (Fig. 4C, left panels) (10). In contrast, the double-mutant ENE effectively protected the A₅₇GA₂ poly(A) tail, which has a single A to G substitution close to its 3' end (Fig. 4C, center panel). The presence of a G residue in the poly(A) tail does not on its own confer resistance to deadenylation, as the A₅₇GA₂ poly(A) tail of a construct lacking the ENE was not protected (Fig. 4C, bottom middle panel). These results, supported by the native gel shift data described above (fig. S8), indicate that the triple helix observed in the structure is critical to ENE function.

Finally, we tested the ability of the double-mutant ENE to protect a poly(A) tail containing a more internal G substitution (A₁₉GA₄₀) (Fig. 4C, right panel). In nuclear extract, this poly(A) tail was rapidly deadenylated to a size consistent with formation of a triple helix that includes the predicted C-G•C base triple (Fig. 4C, right middle panel). The ability of the double-mutant ENE to locate a single G within a long stretch of A nucle-

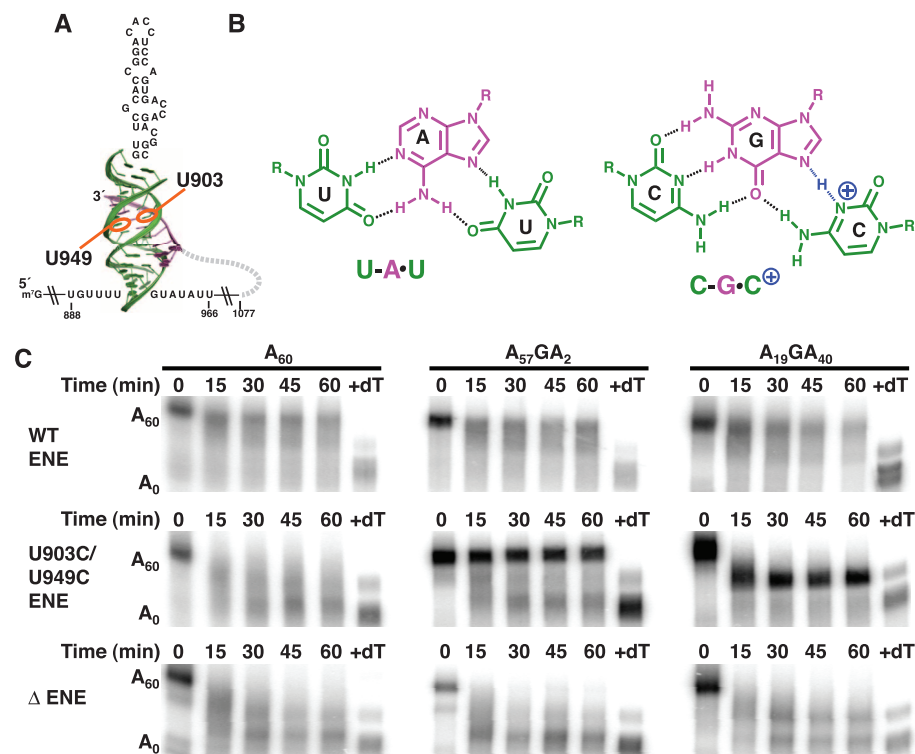


Fig. 4. Triple-helix assembly protects the PAN RNA poly(A) tail from deadenylation. (A) Cartoon of the ENE:A₉ complex structure in the context of full-length PAN, highlighting the locations of U903 and U949 (numbering from the PAN RNA 5' end). (B) Comparison of U-A•U and C-G•C⁺ base triples [colors as in (A), with the hydrogen bond formed upon protonation of the Hoogsteen C nucleotide in blue]. (C) In vitro deadenylation assays show that single G substitutions in the poly(A) tail rescue a nonfunctional double-mutant ENE by formation of C-G•C base triples (24). The substrates consist of the 327-nt 3' terminus of PAN RNA followed by a 60-nt tail either composed of all adenylate (A₆₀) or with single G substitutions 3 or 41 nucleotides from the 3' end of the poly(A) tail (A₅₇GA₂ or A₁₉GA₄₀, respectively); tail identity is designated above the panels. After incubation in HeLa cell nuclear extract (28) for the indicated times, products were separated by denaturing gel electrophoresis. RNAs containing the wild-type ENE, double-mutant (U903C, U949C) ENE, and Δ ENE are shown in the upper, middle, and lower panels, respectively. +dT lanes refer to transcripts in which the poly(A) tail was removed by endogenous ribonuclease H after addition of oligo(dT)₄₀ to the reaction mix. The A₆₀ and A₀ labels on the left show the migration of fully adenylated and deadenylated substrates.

tides is striking. The deadenylation data also demonstrate that the ENE does not require the 3' terminus of the poly(A) tail for binding, despite the involvement of the 3' end of the A₉ RNA in the final base triple of the ENE core:A₉ structure. The results further argue that no specific register is required for the interaction of the wild-type ENE with the PAN RNA poly(A) tail. The presence of multiple binding sites for the ENE along the poly(A) sequence may contribute to its ability to protect tails of various lengths from deadenylation by cellular exonucleases (Fig. 4A). How the ENE may collaborate with poly(A)-binding proteins that are known to coat the poly(A) tails of RNA polymerase II transcripts *in vivo* (25) remains to be determined.

The key feature of the core ENE:A₉ crystal structure is a functionally important U-A•U major-groove triple helix, which is extended by A-minor interactions. The structure reveals an intramolecular clamp mechanism for recognition of poly(A) RNA and suggests how the ENE sequesters the PAN poly(A) tail from degradation by cellular deadenylases. Since viruses routinely borrow strategies from their hosts, we predict that similar mechanisms may protect some cellular noncoding RNAs from rapid turnover.

References and Notes

1. A. Viejo-Borbolla, M. Ottinger, T. F. Schulz, *Curr. HIV/AIDS Rep.* **1**, 5 (2004).
2. E. A. Mesri, E. Cesarman, C. Boshoff, *Nat. Rev. Cancer* **10**, 707 (2010).

3. D. M. Parkin *et al.*, *Lancet Oncol.* **9**, 683 (2008).
4. R. Sun, S. F. Lin, L. Gradoville, G. Miller, *Proc. Natl. Acad. Sci. U.S.A.* **93**, 11883 (1996).
5. W. Zhong, H. Wang, B. Herndier, D. Ganem, *Proc. Natl. Acad. Sci. U.S.A.* **93**, 6641 (1996).
6. M. J. Song, H. J. Brown, T. T. Wu, R. Sun, *J. Virol.* **75**, 3129 (2001).
7. N. K. Conrad, J. A. Steitz, *EMBO J.* **24**, 1831 (2005).
8. N. K. Conrad, S. Mili, E. L. Marshall, M.-D. Shu, J. A. Steitz, *Mol. Cell* **24**, 943 (2006).
9. R. Parker, H. Song, *Nat. Struct. Mol. Biol.* **11**, 121 (2004).
10. A. C. Goldstrohm, M. Wickens, *Nat. Rev. Mol. Cell Biol.* **9**, 337 (2008).
11. N. K. Conrad, M.-D. Shu, K. E. Uyhazi, J. A. Steitz, *Proc. Natl. Acad. Sci. U.S.A.* **104**, 10412 (2007).
12. T. Kiss, E. Fayet-Lebaron, B. E. Jädy, *Mol. Cell* **37**, 597 (2010).
13. Materials and methods are available as supporting material on Science Online.
14. Numbering from the 5' end of the crystallization construct. ENE core nucleotides 1 to 16 correspond to PAN nucleotides 894 to 909, and 23 to 39 correspond to PAN nucleotides 943 to 959.
15. P. Nissen, J. A. Ippolito, N. Ban, P. B. Moore, T. A. Steitz, *Proc. Natl. Acad. Sci. U.S.A.* **98**, 4899 (2001).
16. E. A. Doherty, R. T. Batey, B. Masquida, J. A. Doudna, *Nat. Struct. Mol. Biol.* **8**, 339 (2001).
17. H. Jin, J. P. Loria, P. B. Moore, *Mol. Cell* **26**, 205 (2007).
18. H. Wu, J. Feigon, *Proc. Natl. Acad. Sci. U.S.A.* **104**, 6655 (2007).
19. C. A. Theimer, C. A. Blois, J. Feigon, *Mol. Cell* **17**, 671 (2005).
20. S. D. Gilbert, R. P. Rambo, D. Van Tyne, R. T. Batey, *Nat. Struct. Mol. Biol.* **15**, 177 (2008).
21. G. Felsenfeld, A. Rich, *Biochim. Biophys. Acta* **26**, 457 (1957).
22. G. Felsenfeld, D. Davies, A. Rich, *J. Am. Chem. Soc.* **79**, 2023 (1957).
23. D. J. D'Souza, E. T. Kool, *J. Biomol. Struct. Dyn.* **10**, 141 (1992).

24. The C-G•C base triple is strongest upon protonation of the Hoogsteen C nucleotide, but the triple helical structure can increase the pK_a of the C base, such that full protonation occurs at neutral pH (19).
25. U. Kühn, E. Wahle, *Biochim. Biophys. Acta* **1678**, 67 (2004).
26. N. B. Leontis, E. Westhof, *RNA* **7**, 499 (2001).
27. N.-K. Kim *et al.*, *J. Mol. Biol.* **384**, 1249 (2008).
28. J. D. Dignam, R. M. Lebovitz, R. G. Roeder, *Nucleic Acids Res.* **11**, 1475 (1983).
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Supporting Online Material

www.sciencemag.org/cgi/content/full/330/6008/1244/DC1
Materials and Methods
SOM Text
Figs. S1 to S8
Table S1

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PML Regulates Apoptosis at Endoplasmic Reticulum by Modulating Calcium Release

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The promyelocytic leukemia (PML) tumor suppressor is a pleiotropic modulator of apoptosis. However, the molecular basis for such a diverse proapoptotic role is currently unknown. We show that extranuclear Pml was specifically enriched at the endoplasmic reticulum (ER) and at the mitochondria-associated membranes, signaling domains involved in ER-to-mitochondria calcium ion (Ca²⁺) transport and in induction of apoptosis. We found Pml in complexes of large molecular size with the inositol 1,4,5-trisphosphate receptor (IP₃R), protein kinase Akt, and protein phosphatase 2a (PP2a). Pml was essential for Akt- and PP2a-dependent modulation of IP₃R phosphorylation and in turn for IP₃R-mediated Ca²⁺ release from ER. Our findings provide a mechanistic explanation for the pleiotropic role of Pml in apoptosis and identify a pharmacological target for the modulation of Ca²⁺ signals.

The promyelocytic leukemia gene (*PML*) was originally identified at the breakpoint of the t(15;17) translocation of acute promyelocytic leukemia (APL), and function of the PML protein is frequently lost or aberrant in human solid tumors and hematopoietic malignancies (1, 2). PML is a nuclear protein and an essential component of subnuclear structures termed nuclear bodies (NBs) (3). However, many, if not all, PML

isoforms have shown both cytoplasmic and nuclear localization (4, 5). *Pml*^{-/-} mice and primary cells are protected from apoptosis triggered by a number of diverse stimuli (6).

To determine how PML could regulate such broadly diverse apoptotic responses, we analyzed its intracellular localization by cell fractionation (7, 8). We fractionated homogenates of primary mouse embryonic fibroblasts (MEFs) by ultracentrifugation, focusing on the mitochondria, endoplasmic reticulum (ER), and mitochondria-associated membranes (MAMs), the structures that contain sites where the ER contacts mitochondria. Pml localized both to the nucleus and the cytosol and appeared to localize also to the ER, MAM, and crude mitochondrial fractions but not to "pure" mitochondrial fraction free of ER and nuclear markers (Fig. 1A). These results were confirmed by immunogold labeling of ultrathin cryosections showing that Pml associates with the surface of the ER (Fig. 1B, a and b) and in the proximity of

trifurcation, focusing on the mitochondria, endoplasmic reticulum (ER), and mitochondria-associated membranes (MAMs), the structures that contain sites where the ER contacts mitochondria. Pml localized both to the nucleus and the cytosol and appeared to localize also to the ER, MAM, and crude mitochondrial fractions but not to "pure" mitochondrial fraction free of ER and nuclear markers (Fig. 1A). These results were confirmed by immunogold labeling of ultrathin cryosections showing that Pml associates with the surface of the ER (Fig. 1B, a and b) and in the proximity of

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Fig. 1. Identification of Pml at ER and MAM regions and Ca^{2+} -mediated Pml-dependent cell death. (A) Detection of Pml by immunoblotting in $Pml^{+/+}$ MEFs fractionation. IP₃R, tubulin, proliferating cell nuclear antigen (PCNA), and voltage-dependent anion channel (VDAC) are used as markers. H: homogenate; Mc: crude mitochondria; Mp: pure mitochondria; ER: ER; MAM: C: cytosol; N: nucleus. (B) Immunogold labeling of Pml near the rough ER (r), mitochondria (m), and MAM (arrowheads) in $Pml^{+/+}$ MEFs. Gold particles (15 nm) are mostly associated with the surface of the ER (7.07 gold particles/ μm^2) and more occasionally with mitochondrial membranes (3.08 gold particles/ μm^2) (a and b). Specificity of the antibodies is demonstrated by labeling of nuclear bodies (n) (c). Morphologically identified MAM often demonstrated labeling at contacts between ER and mitochondria [(d) to (g), and arrowheads in insets therein]. Insets correspond to boxed areas. Bar: (a) 360 nm; (b) 340 nm; (c) 370 nm; (d) 188 nm, inset 120 nm; (e) 260 nm, inset 190 nm; (f) 340 nm, inset 180 nm; (g) 280 nm,

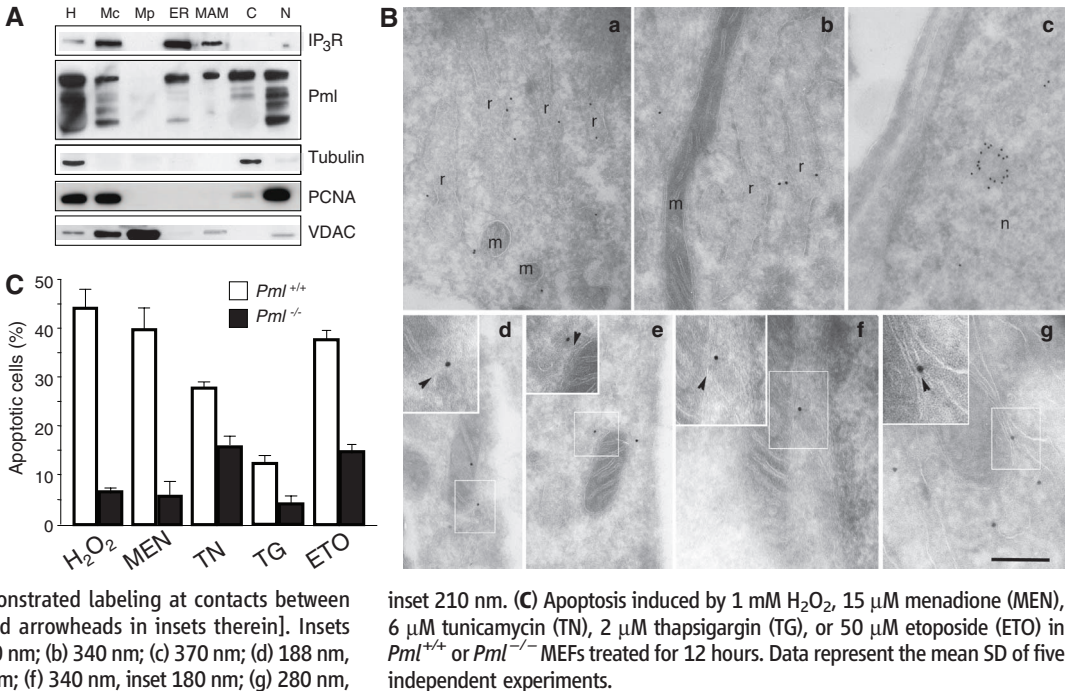
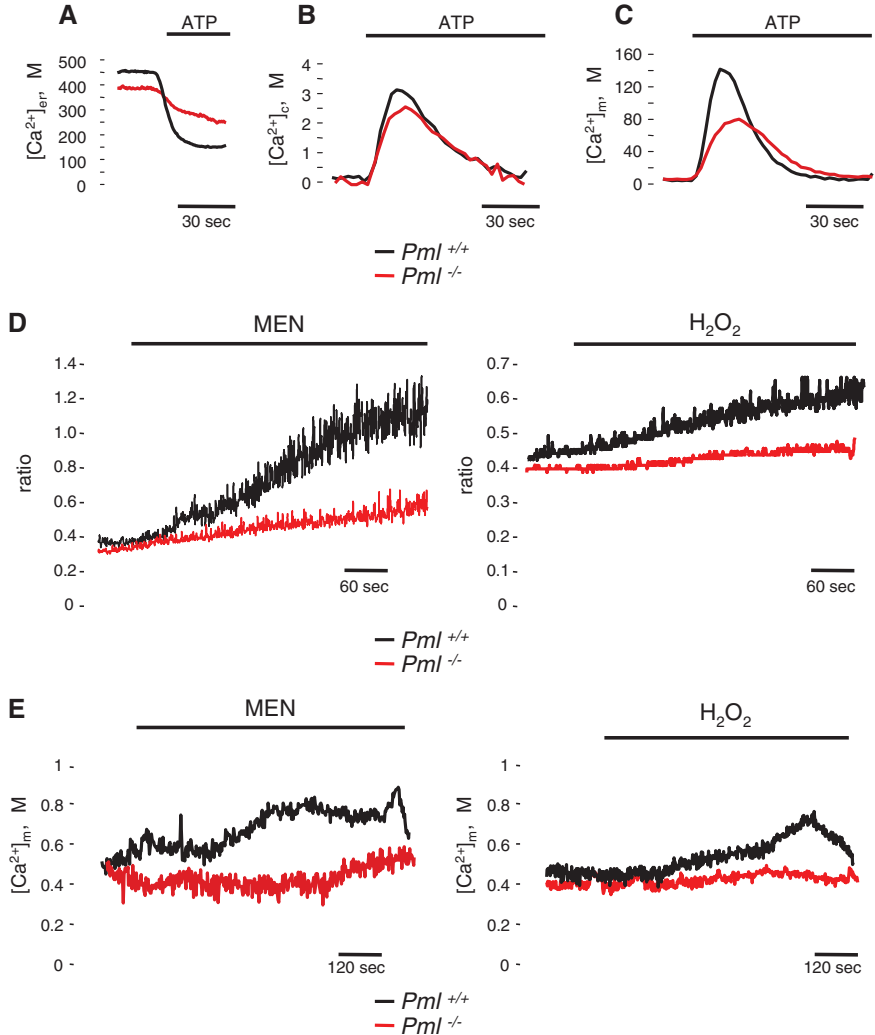


Fig. 2. Intracellular Ca^{2+} homeostasis in $Pml^{+/+}$ and $Pml^{-/-}$ MEFs. (A to C) ER (A), cytosolic (B), and mitochondrial (C) Ca^{2+} homeostasis measurements with aequorins. Where indicated, cells were treated with 100 μM ATP. $Pml^{+/+}$: $[\text{Ca}^{2+}]_{\text{ER}}$ peak $448 \pm 32 \mu\text{M}$; $[\text{Ca}^{2+}]_{\text{c}}$ peak $3.3 \pm 0.16 \mu\text{M}$; $[\text{Ca}^{2+}]_{\text{m}}$ peak $138 \pm 14 \mu\text{M}$. $Pml^{-/-}$: $[\text{Ca}^{2+}]_{\text{ER}}$ peak $386 \pm 42 \mu\text{M}$; $[\text{Ca}^{2+}]_{\text{c}}$ peak $2.65 \pm 0.23 \mu\text{M}$; $[\text{Ca}^{2+}]_{\text{m}}$ peak $78 \pm 10 \mu\text{M}$. $n = 15$ samples from five independent experiments, $P < 0.01$. (D) MEFs loaded with calcium-sensitive fluorescent dye fura-2 were stimulated with menadione (MEN) or H_2O_2 . The kinetic behavior of the $[\text{Ca}^{2+}]_{\text{c}}$ response is presented as the ratio of fluorescence at 340 nm/380 nm. In these, and other fura-2 experiments, the traces are representative of at least 10 single-cell responses from three independent experiments. (E) Analysis of $[\text{Ca}^{2+}]_{\text{m}}$ during oxidative stress. Where indicated, cells were stimulated with 30 μM MEN or 2 mM H_2O_2 . $n = 10$ samples from three independent experiments.



the mitochondrial membrane at contact sites between the ER and mitochondria (Fig. 1B, d to g).

In view of the localization of Pml at the ER and MAM, we investigated its requirement in apoptosis induced by ER stress (9). Matched wild-type (*Pml*^{+/+}) and *Pml*^{-/-} MEFs were treated with ER stress inducers: H₂O₂ and menadione (MEN), two oxidizing agents that induce ER Ca²⁺ release; tunicamycin (TN) or an inhibitor of protein N-glycosylation; and thapsigargin (TG), an inhibitor of the sarcoplasmic/ER Ca²⁺-ATPase (adenosine triphosphatase). After 12 hours of treatment, the percentage of apoptotic cells in *Pml*^{-/-} MEFs was much lower than that observed in *Pml*^{+/+} MEFs under all treatment conditions (Fig. 1C and fig. S1).

MAMs are specialized domains selectively enriched in critical Ca²⁺ signaling elements, which mediate Ca²⁺ transfer between ER and mitochondria (10, 11), such as the inositol 1,4,5-trisphosphate receptor (IP₃R) (12). Ca²⁺ signaling has a major role in the regulation of cell death (13, 14). Release of the ER Ca²⁺ pool through the type 3 IP₃R

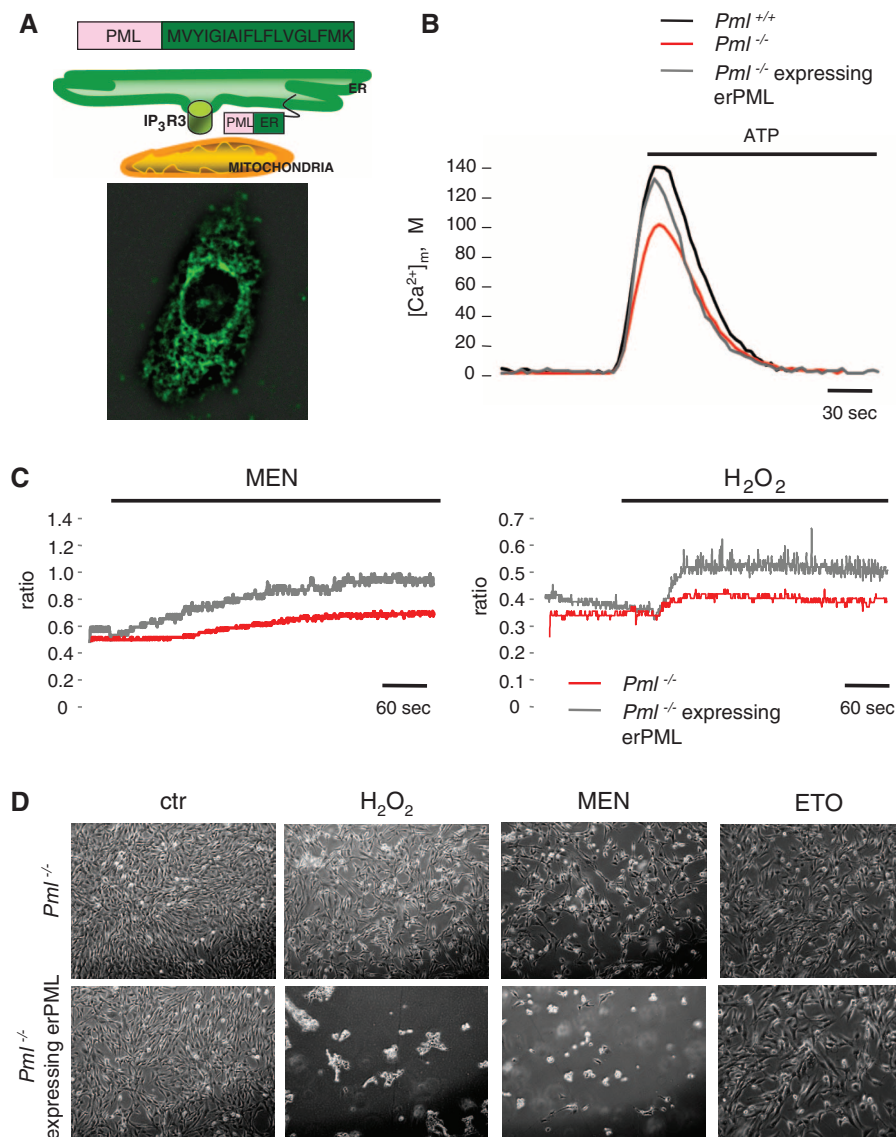
(IP₃R) appears to induce a sensitization of cells to apoptotic stimuli (15, 16).

To investigate the role of Pml in Ca²⁺ homeostasis, we used recombinant Ca²⁺-sensitive bioluminescent protein aequorin (17). In *Pml*^{+/+} MEFs, the Ca²⁺ concentration ([Ca²⁺]_{ER}) in the lumen of the ER ([Ca²⁺]_{ER}) at steady state was ~450 μM, whereas in *Pml*^{-/-} MEFs it was lower. When the cells were stimulated with adenosine 5'-triphosphate (ATP), the P2Y receptor agonist that causes release of Ca²⁺ from the ER, the decreases in the [Ca²⁺]_{ER} observed in *Pml*^{+/+} MEFs in quantitative and kinetic terms were larger and faster than in *Pml*^{-/-} MEFs, reflecting a more rapid flow of Ca²⁺ through the IP₃R (Fig. 2A). In turn, the [Ca²⁺]_{ER} increases evoked by stimulation with ATP in the cytosol ([Ca²⁺]_c) and mitochondria ([Ca²⁺]_m) were smaller in *Pml*^{-/-} than in *Pml*^{+/+} MEFs (Fig. 2, B and C, and fig. S2). A mitochondrial Ca²⁺ deregulation was observed also in human-derived cells in which PML was depleted (fig. S3) and in different cellular models of APL (fig. S4).

We then investigated whether the absence of Pml could alter the increases in [Ca²⁺]_c and [Ca²⁺]_m induced by apoptotic stimuli. In *Pml*^{-/-} MEFs, the increases in [Ca²⁺]_c and [Ca²⁺]_m evoked by the oxidative apoptotic stimuli, such as MEN and H₂O₂ that trigger both a progressive release of Ca²⁺ from the ER and an activation of the capacitative Ca²⁺ influx (18), were smaller as mentioned above (Fig. 2, D and E).

To determine whether the effects of Pml on regulation of Ca²⁺ homeostasis depend on its localization to the ER and MAMs, we generated a chimeric protein containing the entire PML protein that was targeted to the outer surface of the ER (19). This chimera, designated erPML, localized to the ER and MAMs in *Pml*^{-/-} MEFs, as revealed by immunocytochemical staining, immunogold labeling, and subfractionation (Fig. 3A and fig. S5). The introduction of erPML in *Pml*^{-/-} MEFs restored Ca²⁺ signals evoked by either agonist (Fig. 3B and fig. S6) or apoptotic stimuli (MEN or H₂O₂) (Fig. 3C) to values comparable to

Fig. 3. erPML chimera reestablishes the [Ca²⁺]_m and apoptotic responses in *Pml*^{-/-} MEFs. **(A)** Schematic map of the erPML chimera and immunofluorescence image, stained with the antibody to PML, of *Pml*^{-/-} MEFs expressing erPML. **(B)** erPML reestablishes the agonist-dependent [Ca²⁺]_m response in *Pml*^{-/-} MEFs ([Ca²⁺]_m peak 135 ± 12 μM) to values comparable to those of *Pml*^{+/+} MEFs. **(C)** *Pml*^{-/-} and *Pml*^{-/-} MEFs expressing erPML previously incubated with fura-2 were stimulated with menadione (MEN) or H₂O₂. **(D)** Representative microscopic fields of *Pml*^{-/-} MEFs and *Pml*^{-/-} expressing erPML before and after treatment with 1 mM H₂O₂, 15 μM MEN, or 50 μM etoposide (ETO) for 16 hours.



those in *Pml*^{+/+} MEFs (Fig. 2, C and D). This effect was associated with a reestablished sensitivity to apoptosis induced by ER stress but did not restore the sensitivity to etoposide (ETO) (Fig. 3D and fig. S7A), a DNA-damaging agent that triggers apoptotic death by a Ca²⁺-independent process (fig. S7B). Overall, these experiments indicate that the absence of Pml causes a reduction in the amplitude of Ca²⁺ signals induced by ATP, other agents, or apoptotic stimuli, and that forcing PML to the ER rescues these defects. A PML protein targeted to the nucleus restored the formation of NBs, but did not restore the Ca²⁺ responses and the sensitivity to ER stress-dependent cell death, although it restored response to other apoptotic stimuli such as ETO (fig. S8).

To investigate the mechanism underlying these activities of Pml, we tested whether Pml could functionally and physically interact with the IP₃R. Immunoprecipitation of IP₃R led to the coprecipitation of Pml (Fig. 4A and fig. S9) and vice versa (fig. S10). Amounts of phosphorylated-

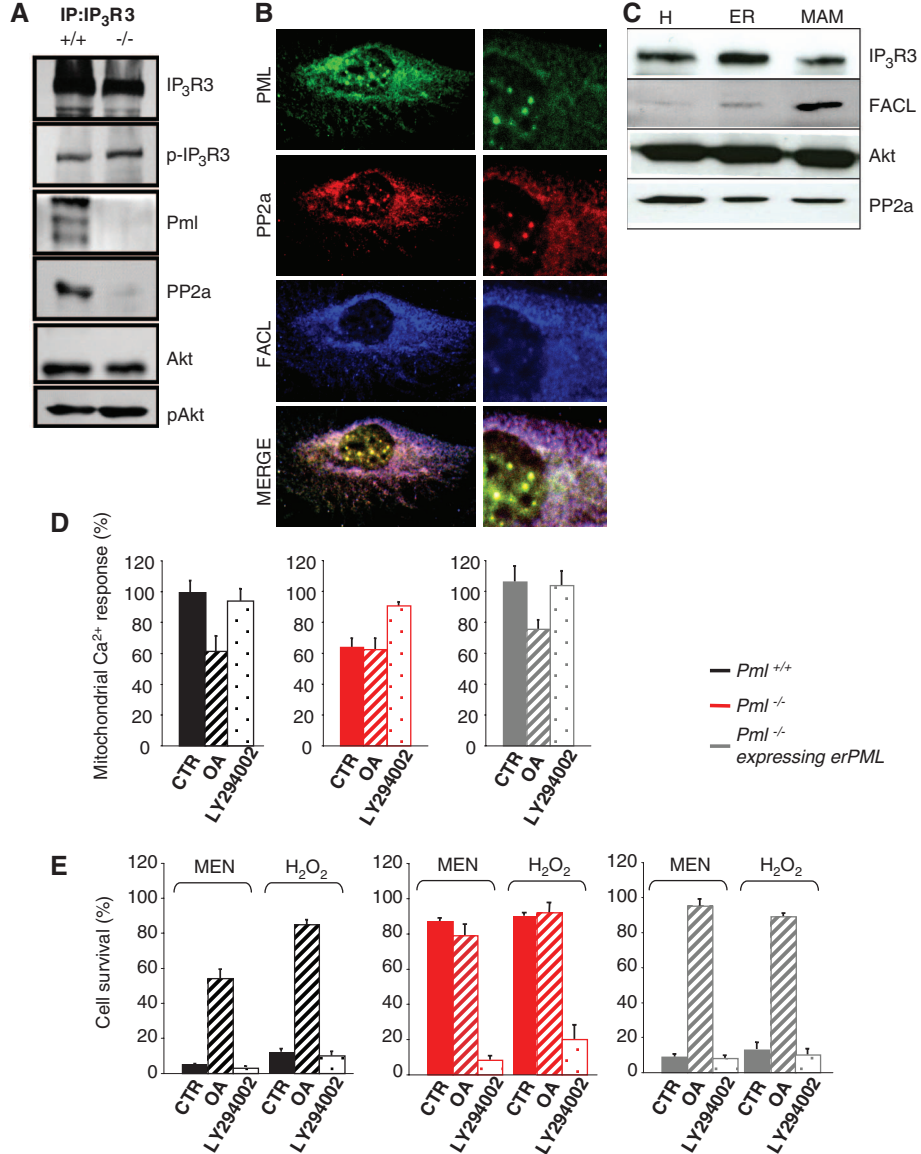
IP₃R (p-IP₃R) were higher in *Pml*^{-/-} than in *Pml*^{+/+} MEFs (Fig. 4A and fig. S11).

Reduced cellular sensitivity to apoptotic stimuli was observed in cells with high activity of the protein kinase Akt, as a result of diminished Ca²⁺ flux from the ER through the IP₃R (20, 21). The amount of phosphorylated Akt (pAkt) (that is, the active form of Akt) coprecipitated with IP₃R (Fig. 4A and fig. S9) was higher in *Pml*^{-/-} than in *Pml*^{+/+} MEFs (fig. S11). Dephosphorylation of Akt at the MAM might occur through Pml-mediated recruitment of the phosphatase PP2a. Indeed, Pml interacts with PP2a in Pml-NBs (22). Further, the amount of PP2a coprecipitated with IP₃R (Fig. 4A and figs. S9 and S10) was diminished in *Pml*^{-/-} MEFs (Fig. 4A and fig. S11). Thus, in the absence of *Pml*, reduced Ca²⁺ release could be caused by increased phosphorylation and activation of Akt at the ER due to an impaired PP2a activity, which in turn impair Ca²⁺ flux through the IP₃R because of its hyperphosphorylated state (figs. S11 and S12).

To determine whether IP₃R, Pml, Akt, and PP2a interact in a complex, we next performed two-dimensional blue native analysis. We found that Pml, IP₃R, Akt, and PP2a colocalize in high molecular weight complexes, supporting their possible interaction in the native state (fig. S13). Finally, we demonstrated the localization of all these proteins at the ER and MAM through immunocytochemical staining and subfractionation (Fig. 4, B and C, and fig. S14).

We further investigated the correlation among Pml, Akt, and PP2a at the ER and the regulation of the IP₃R by a selective inhibition of either Akt or PP2a. Pretreatment of cells with okadaic acid (OA, a PP2a inhibitor) caused a reduction in [Ca²⁺]_m responses to ATP stimulation and a reduced H₂O₂- or MEN-induced death in *Pml*^{+/+} MEFs and in *Pml*^{-/-} MEFs expressing erPML, but not in *Pml*^{-/-} MEFs (Fig. 4, D and E, and figs. S15, S16, and S18), in which PP2a activity is impaired. LY294002 (an inhibitor of Akt) had no

Fig. 4. Modulation of [Ca²⁺]_m and apoptotic responses by Pml through Akt- and PP2a-dependent phosphorylation of IP₃R. **(A)** Coimmunoprecipitation of IP₃R with Pml, Akt, and PP2a in *Pml*^{+/+} MEFs. In the same blot, the levels of p-IP₃R and pAkt are shown. **(B)** Localization of Pml (green) and PP2a (red) at ER and MAM sites in *Pml*^{+/+} MEFs analyzed by immunofluorescence. FACL [long-chain fatty acid-CoA (coenzyme A) ligase type 4, blue] was used as MAM marker. **(C)** *Pml*^{+/+} MEFs subcellular fractionation and identification of PP2a and Akt at ER and MAM fractions by immunoblot. **(D)** Effects of okadaic acid (OA, 1 μM for 1 hour) and LY294002 (5 μM for 30 min) on agonist-dependent [Ca²⁺]_m responses in *Pml*^{+/+}, *Pml*^{-/-}, and *Pml*^{-/-} MEFs expressing erPML. [Ca²⁺]_m is represented as a percentage of the peak value of control cells. Representative traces are shown in fig. S15. **(E)** Quantification of cell survival of *Pml*^{+/+}, *Pml*^{-/-}, and *Pml*^{-/-} MEFs expressing erPML, control (CTR, untreated) and treated first with OA (1 μM for 1 hour) or LY294002 (5 μM for 30 min) and then H₂O₂ or menadione (MEN) for 16 hours. The data show the percentage of living cells in the whole-cell population negative for annexin-V-fluorescein isothiocyanate and propidium iodide staining, analyzed by flow cytometry. Data show the means SD from three independent experiments.



effect on the agonist-dependent $[Ca^{2+}]_m$ transients and on apoptosis in $Pml^{+/+}$ or $Pml^{-/-}$ MEFs expressing erPML , whereas it increased agonist-dependent $[Ca^{2+}]_m$ responses and restored sensitivity to H_2O_2 or MEN (Fig. 4, D and E, and figs. S15, S17, and S18) in $Pml^{-/-}$ MEFs (in which high levels of pAkt are observed; Fig. 4A and fig. S11). These results were confirmed in experiments in which RNA interference was used to deplete cells of Akt or PP2a proteins (fig. S19, A and B) or a constitutively active form of Akt (m/p Akt) was expressed (fig. S19C).

Our data highlight an extranuclear, transcription-independent function of Pml that regulates cell survival through changes in Ca^{2+} signaling in the ER, cytosol, and mitochondria (fig. S20). This effect appears to be specific to Ca^{2+} -mediated apoptotic stimuli because alteration in Pml did not influence cell death in cells treated with ETO, which activates the apoptotic pathway in a way largely independent of Ca^{2+} .

This mechanism may explain how Pml can so broadly regulate the early (and transcription independent) apoptotic response. Our findings may have implications in tumorigenesis where the function of Pml is frequently lost, or in other patho-

physiological conditions where Pml is accumulated such as cell stress, or infection with viral or bacterial pathogens.

References and Notes

1. P. Salomoni, P. P. Pandolfi, *Cell* **108**, 165 (2002).
2. C. Gurrieri *et al.*, *J. Natl. Cancer Inst.* **96**, 269 (2004).
3. T. H. Shen, H. K. Lin, P. P. Scaglioni, T. M. Yung, P. P. Pandolfi, *Mol. Cell* **24**, 331 (2006).
4. H. K. Lin, S. Bergmann, P. P. Pandolfi, *Nature* **431**, 205 (2004).
5. W. Condemine *et al.*, *Cancer Res.* **66**, 6192 (2006).
6. R. Bernardi, A. Papa, P. P. Pandolfi, *Oncogene* **27**, 6299 (2008).
7. M. R. Wieckowski, C. Giorgi, M. Lebedzinska, J. Duszyński, P. P. Pandolfi, *Nat. Protoc.* **4**, 1582 (2009).
8. Materials and methods are available as supporting material on Science Online.
9. C. Giorgi, D. De Stefani, A. Bononi, R. Rizzuto, P. P. Pandolfi, *Int. J. Biochem. Cell Biol.* **41**, 1817 (2009).
10. P. P. Pandolfi, C. Giorgi, R. Siviero, E. Zecchini, R. Rizzuto, *Oncogene* **27**, 6407 (2008).
11. T. Hayashi, R. Rizzuto, G. Hajnoczky, T. P. Su, *Trends Cell Biol.* **19**, 81 (2009).
12. R. L. Patterson, D. Boehning, S. H. Snyder, *Annu. Rev. Biochem.* **73**, 437 (2004).
13. C. Giorgi, A. Romagnoli, P. P. Pandolfi, R. Rizzuto, *Curr. Mol. Med.* **8**, 119 (2008).
14. D. E. Clapham, *Cell* **131**, 1047 (2007).
15. P. P. Pandolfi, R. Rizzuto, *Cell Death Differ.* **13**, 1409 (2006).
16. C. C. Mendes *et al.*, *J. Biol. Chem.* **280**, 40892 (2005).
17. P. P. Pandolfi, A. Rimesi, A. Romagnoli, A. Prandini, R. Rizzuto, *Methods Cell Biol.* **80**, 297 (2007).
18. P. P. Pandolfi *et al.*, *EMBO J.* **20**, 2690 (2001).
19. M. Yang, J. Ellenberg, J. S. Bonifacio, A. M. Weissman, *J. Biol. Chem.* **272**, 1970 (1997).
20. T. Szado *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **105**, 2427 (2008).
21. S. Marchi *et al.*, *Biochem. Biophys. Res. Commun.* **375**, 501 (2008).
22. L. C. Trotman *et al.*, *Nature* **441**, 523 (2006).
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Supporting Online Material

www.sciencemag.org/cgi/content/full/science.1189157/DC1
Materials and Methods
Figs. S1 to S20
References

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Reprogramming Cellular Behavior with RNA Controllers Responsive to Endogenous Proteins

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Synthetic genetic devices that interface with native cellular pathways can be used to change natural networks to implement new forms of control and behavior. The engineering of gene networks has been limited by an inability to interface with native components. We describe a class of RNA control devices that overcome these limitations by coupling increased abundance of particular proteins to targeted gene expression events through the regulation of alternative RNA splicing. We engineered RNA devices that detect signaling through the nuclear factor κB and Wnt signaling pathways in human cells and rewire these pathways to produce new behaviors, thereby linking disease markers to noninvasive sensing and reprogrammed cellular fates. Our work provides a genetic platform that can build programmable sensing-actuation devices enabling autonomous control over cellular behavior.

Cellular decisions, such as differentiation, response to stress, disease progression, and apoptosis, depend on regulatory networks that control enzymatic activities, protein translocation, and genetic responses. Central to the genetic programming of biological systems is the ability to process information within cellular networks and link this information to new cellular behaviors, in essence rewiring network to-

pologies. Altered network topologies have been achieved through engineered transcriptional networks (1, 2) and signal transduction cascades (3). However, these systems are limited to processing transcription-factor inputs, which represent a small fraction of the human proteome (4, 5) or require replacing endogenous cellular components. Alternative platforms for constructing sensing-actuation devices based on the detection of broad classes of proteins will have widespread applications in basic research, biotechnology, and medicine.

RNA is a promising substrate for platforms to interface with cellular networks because of the versatile sensing and actuation functions that RNA can exhibit and the ease with which RNA structures can be designed (6, 7). RNA-based

sensing-actuation devices have been engineered that respond predominantly to externally applied small-molecule (6, 8, 9) and nucleic acid (10–12) inputs and control gene expression through diverse mechanisms. Pre-mRNA splicing is one such mechanism, in which devices responsive to exogenous small-molecule and protein inputs can regulate splicing events (8, 13). However, protein-responsive gene regulatory platforms based on programmed alternative splicing must support modular and extensible input/output functionalities, provide regulatory properties that translate to control over cell behaviors, and be sensitive to changes in endogenous protein concentrations or localization. Although RNA aptamers that bind to proteins have been generated through in vitro selection methods (14, 15), such protein-sensing components have not been routinely integrated into RNA-based regulatory devices, leaving a large number of biological signals currently inaccessible.

We developed a protein-responsive RNA-based regulatory device by integrating RNA aptamers that bind to protein ligands in key intronic locations of an alternatively spliced transcript, thus linking intracellular protein concentrations to gene-expression events (16). Our regulatory platform consists of an output module, or a gene of interest (GOI) placed downstream of the sensing-actuation device, and a three-exon, two-intron mini-gene in which the middle exon is alternatively spliced or excluded (Fig. 1A). The middle exon contains a stop codon, such that expression of the GOI is high when the exon is excluded. Control is exerted by the input module, composed of an RNA aptamer that senses changes in nuclear protein concentrations whereby ligand binding to the

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aptamer alters the splicing pattern, probably through steric hindrance or recruitment of components involved in spliceosome site (ss) recognition (Fig. 1, A and B) (17). We systematically analyzed the device architecture to determine the intron positions that enabled aptamer-mediated protein-responsive regulation of alternative splicing. We inserted the aptamer for the bacteriophage coat protein MS2 (18) at six positions in each intron (1 to 12) of the SMN1 minigene (Fig. 1C and fig. S1A) (19) and linked the device to the gene encoding green fluorescent protein (GFP). We selected the SMN1

minigene because key regulatory sequences are located in its exon regions (20), such that insertion of synthetic sequences into intronic regions is not likely to strongly affect splicing patterns. Human embryonic kidney–293 cell lines that stably expressed these devices or the corresponding negative controls expressing mutant aptamers (SOM text S1) displayed differences in fluorescence compared with cells expressing a device containing no aptamer sequence, with insertion at most positions causing increased exon exclusion (fig. S1, B and C), indicating that secondary structure can modulate splicing patterns.

To examine protein-specific effects on splicing, we transfected cell lines with a plasmid encoding the MS2 coat protein fused to fluorescent protein DsRed and simian virus 40 nuclear localization signal (MS2-DsRed) (Fig. 1C and fig. S1D). Integration of aptamers into six positions resulted in increases in fluorescence ($P < 0.05$, Student's t test), and integration into three positions resulted in decreases in fluorescence ($P < 0.05$) relative to that of cell lines expressing DsRed. Control experiments showed that these effects were specific to the wild-type (WT) aptamer (Fig. 1, D and E, fig. S1E, and table S1). Transcript isoform analysis

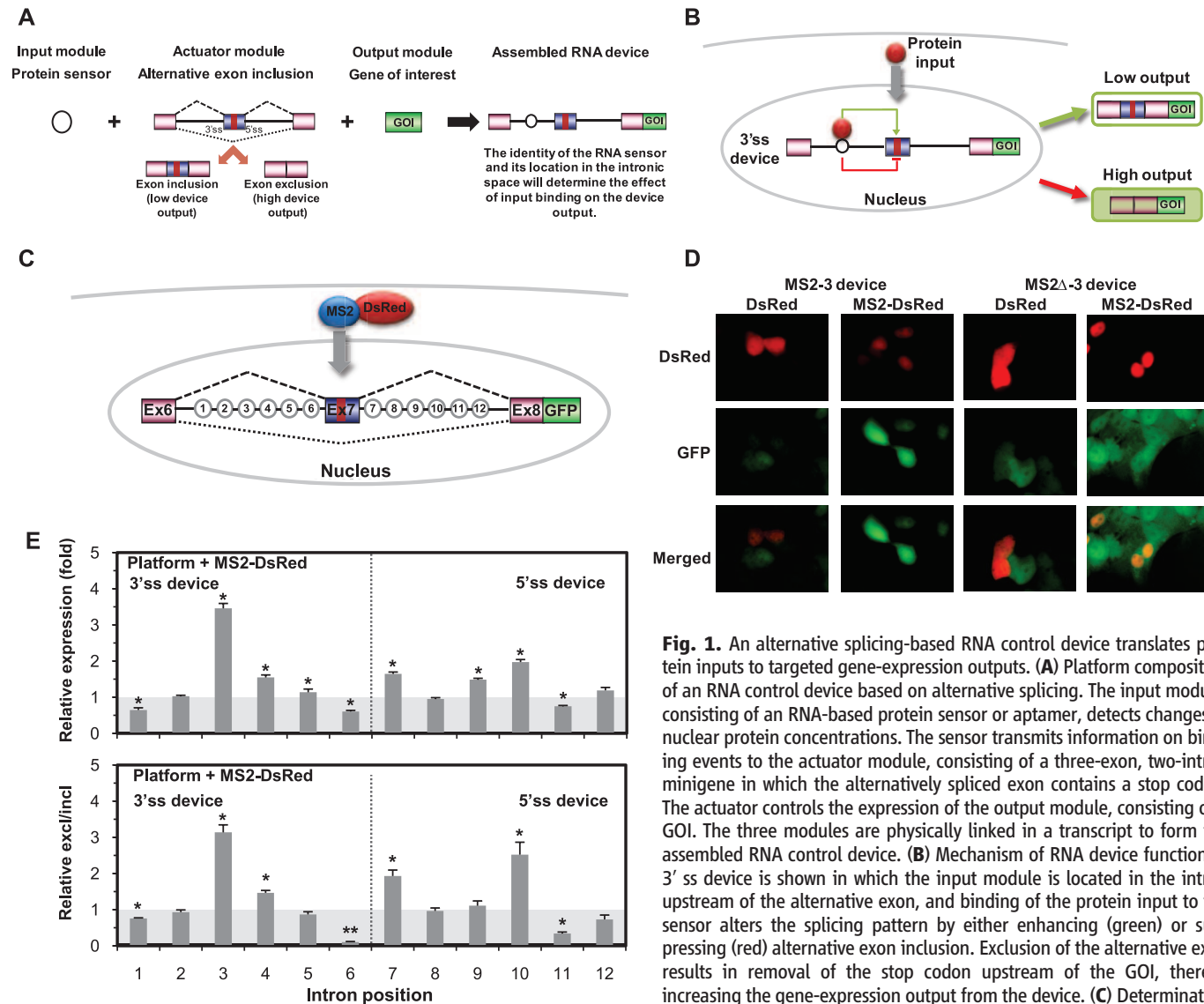


Fig. 1. An alternative splicing-based RNA control device translates protein inputs to targeted gene-expression outputs. **(A)** Platform composition of an RNA control device based on alternative splicing. The input module, consisting of an RNA-based protein sensor or aptamer, detects changes in nuclear protein concentrations. The sensor transmits information on binding events to the actuator module, consisting of a three-exon, two-intron minigene in which the alternatively spliced exon contains a stop codon. The actuator controls the expression of the output module, consisting of a GOI. The three modules are physically linked in a transcript to form the assembled RNA control device. **(B)** Mechanism of RNA device function. A 3' ss device is shown in which the input module is located in the intron upstream of the alternative exon, and binding of the protein input to the sensor alters the splicing pattern by either enhancing (green) or suppressing (red) alternative exon inclusion. Exclusion of the alternative exon results in removal of the stop codon upstream of the GOI, thereby increasing the gene-expression output from the device. **(C)** Determination of optimal input-module location within intronic sequence space of the

regulatory device. The MS2 aptamer was inserted at 12 intronic positions spaced by 15 nucleotides flanking the alternatively spliced exon. **(D)** Fluorescence images of the MS2-responsive devices. The increased fluorescence output from an MS2-responsive device is specific to the MS2-DsRed protein input and the WT MS2 aptamer in position 3 (MS2-3). MS2Δ-3, mutant MS2 aptamer in position 3. **(E)** The response of the MS2-responsive device to the MS2-DsRed protein is affected by the location of the input module. For all activities reported as relative expression (fold), the ratio of the mean GFP levels of the WT RNA device in the presence of ligand (MS2-DsRed) to the absence of ligand (DsRed) is normalized to the same ratio for the mutant device. Transcript isoform analysis of the MS2-responsive devices with qRT-PCR supported the gene-expression data (bottom panel). For all qRT-PCR data reported as relative exclusion/inclusion (excl/incl) (fold), the ratio of the mean expression levels of the exon-7 excluded isoform to the exon-7 included isoform for the WT device in the presence of ligand to the absence of ligand is normalized to the same ratio for the mutant device. For all reported activities, mean expression levels from two independent experiments are shown. Error bars represent $\pm$ SD from mean values. P values derived from the Student's t test are as follows: $*P < 0.05$ and $**P < 0.01$. Unnormalized expression levels for all devices are provided in tables S1 to S6.

by quantitative real-time polymerase chain reaction (qRT-PCR) confirmed the effect on splicing (Fig. 1E and fig. S1F), and there was a significant correlation between splicing patterns and fluorescence ($P < 0.01$, analysis of variance). Although the regulatory effects of the devices were modest (~two- to fourfold), these effects are comparable to those in other splicing (21) and RNA regulatory (22) systems that have key roles in controlling biological processes. We selected positions 3, 6, and 10 as points of input-module integration for device tailoring on the basis of their relative amount of protein-mediated splicing regulation and location relative to splicing motifs (3' ss, 5' ss, branch point, and polypyrimidine tract).

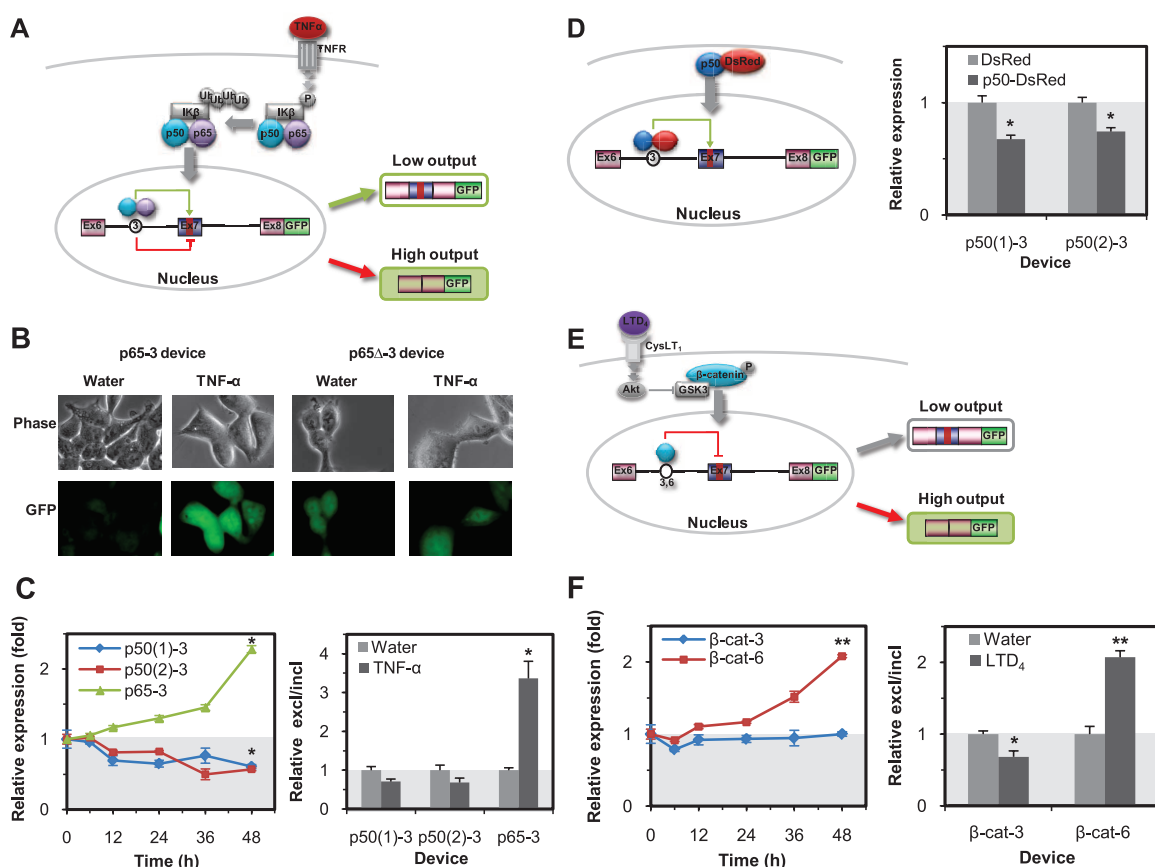
To investigate the modularity of the input processing function of our RNA devices and the ability to detect nuclear localized proteins resulting from activated signaling pathways, we built devices with aptamers that bind the subunits p50 (23, 24) and p65 (25) of the transcription factor NF- κ B inserted into position 3 (Fig. 2A). NF- κ B

p50 and p65 dimers have an important role in disease by binding to κ B sites in promoters or enhancers of genes participating in immune and inflammatory responses, cell adhesion, proliferation, and apoptosis (25). We induced NF- κ B signaling and subsequent translocation of p50 and p65 to the nucleus in cell lines stably expressing the NF- κ B devices with tumor necrosis factor- α (TNF- α) (26). The p65-3 device displayed increased gene expression ($P < 0.01$), corresponding to an increase in exon exclusion as a result of p65 binding to the sensor, whereas the p50-3 devices exhibited decreased gene expression ($P < 0.05$) and exon exclusion as a result of p50 binding to the sensor (Fig. 2, B and C, fig. S2A, and table S2). Controls with mutant aptamer devices showed that responses were specific to the WT aptamer sequences. Cell lines expressing the p50-responsive devices and a p50-DsRed fusion exhibited decreases in fluorescence when compared with cells expressing DsRed (Fig. 2D, fig. S2B, and table S3), indicating that the response ob-

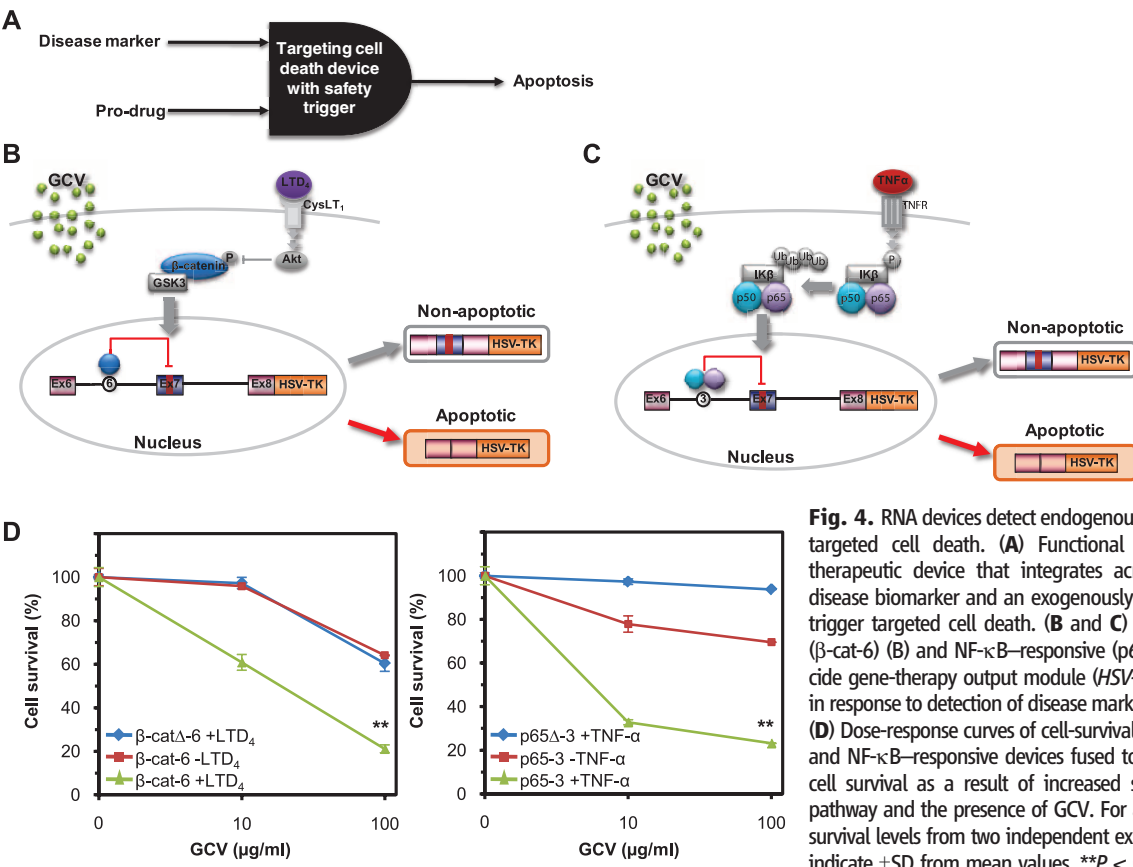
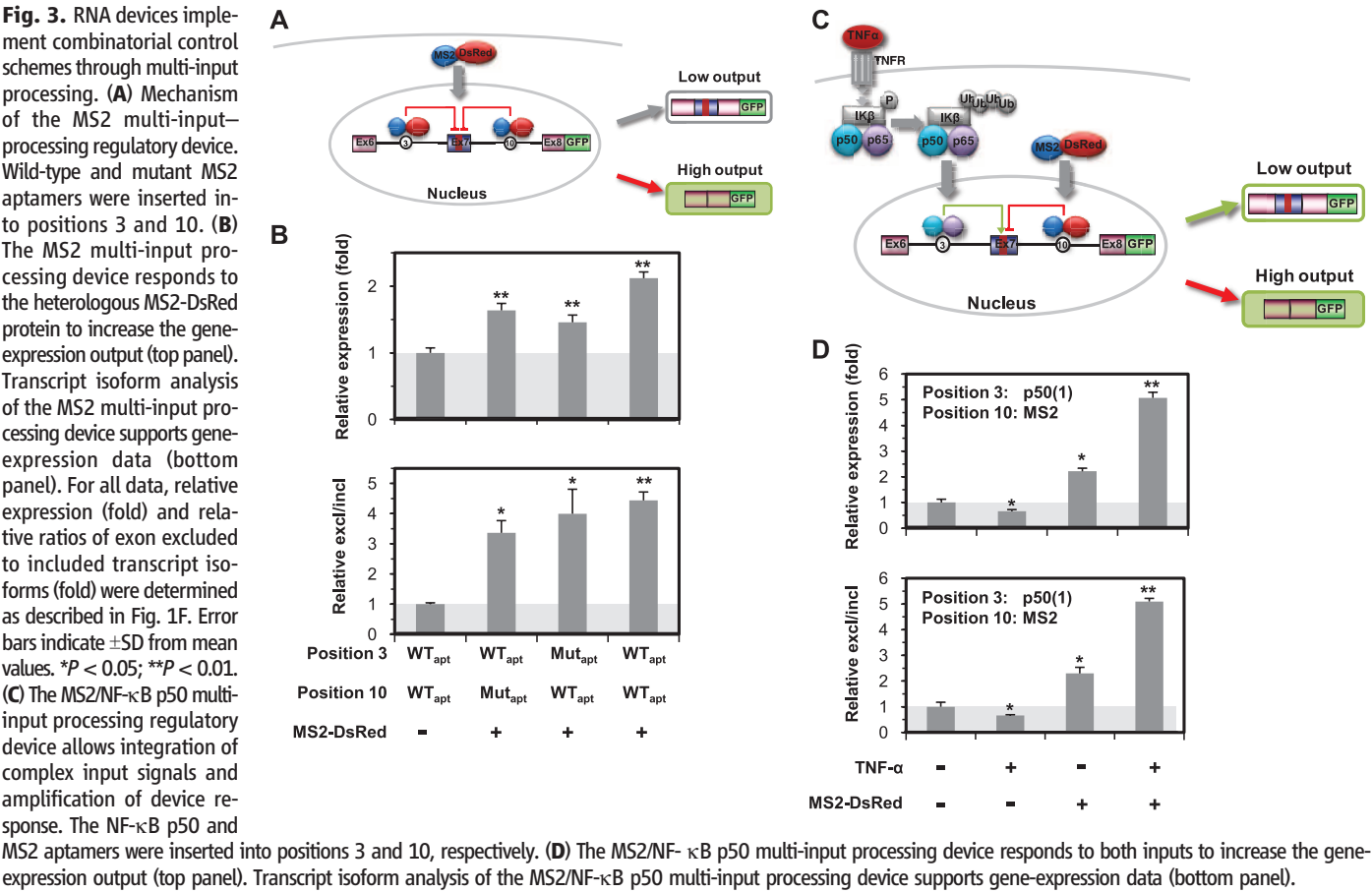
served under TNF- α stimulation is directly mediated by p50 binding. The differing output signals from the p65- and p50-responsive devices may be due to differences in aptamer binding (25), aptamer structure, or interactions of p65 and p50 with spliceosomal components (SOM text S2).

We also inserted aptamers that recognize the signaling protein β -catenin (27) into sites 3 and 6 to build β -catenin-responsive devices (Fig. 2E). β -catenin is a central component of the Wnt signaling pathway and is localized to the nucleus upon pathway activation to aid in the transcription of genes that regulate cell growth, differentiation, and tumorigenesis (27). We examined the effect of stimulating the β -catenin pathway with leukotriene D₄ (LTD₄) on the response of our engineered β -catenin-responsive devices. The β -cat-6 device exhibited increased gene expression ($P < 0.05$), corresponding to an increase in exon exclusion (Fig. 2F, fig. S2C, and table S4), whereas the β -cat-3 device did not respond to LTD₄ stimulation. Control experiments demon-

Fig. 2. RNA control devices detect endogenous protein inputs and signaling through native pathways. (A) Mechanism of the NF- κ B-responsive device based on TNF- α stimulation (20 ng/ml) of the NF- κ B pathway. Ligand binding to the TNF- α receptor leads to activated signaling and translocation of p50 and p65 into the nucleus. The NF- κ B-responsive devices contain NF- κ B p65 (p65-3) or p50 [p50(1)-3 and p50(2)-3] aptamers inserted into position 3. TNFR, TNF- α receptor; P, phosphorylated; Ub, ubiquitination; IK β , inhibitor of NF- κ B. (B) Phase (top) and fluorescence (bottom) images of the NF- κ B p65-responsive devices. The increased fluorescence output from a NF- κ B-responsive device is specific to the pathway stimulation and the WT p65 aptamer in position 3 (p65-3). (C) NF- κ B-responsive devices exhibited responses to TNF- α stimulation at the level of gene expression (left panel) and splicing pattern (right panel). For all data, relative expression (fold) was determined as described in Fig. 1F. qRT-PCR data are reported as relative excl/incl, the ratio of the mean expression levels of the exon-7 excluded isoform to the exon-7 included isoform for the WT device relative to the same ratio for the mutant device under the indicated ligand condition. Error bars indicate $\pm$ SD from mean values. * $P < 0.05$. (D) Mechanism of the NF- κ B p50-responsive device based on a p50-DsRed protein input and corresponding device response. The NF- κ B p50-responsive devices exhibited responses to a heterologous p50-DsRed protein similar to that observed with TNF- α stimulation. Activities were reported as



relative expression by taking the ratio of the mean GFP levels of the WT RNA device to that from the mutant device under the indicated ligand condition. Error bars indicate $\pm$ SD from mean values. * $P < 0.05$. (E) Mechanism of the β -catenin-responsive device based on LTD₄ stimulation (80 nM) of the Wnt pathway. LTD₄ stimulation leads to stabilization of β -catenin and accumulation in the nucleus. The β -catenin-responsive devices contain the β -catenin aptamer in positions 3 (β -cat-3) and 6 (β -cat-6). Akt, serine/threonine protein kinase; GSK3, glycogen synthase kinase 3; CysLT₁, cysteinyl leukotriene receptor. (F) β -catenin-responsive devices exhibited responses to LTD₄ stimulation at the level of gene expression (left panel) and splicing pattern (right panel).



strated that the β -cat-6 device response was specific to the WT aptamer sequences. Results from the MS2, NF- κ B, and β -catenin studies demonstrate that particular protein ligands can have distinct positional and functional effects on splicing, such that aptamer position and the target protein provide device tuning capability. These studies verify the flexibility of our synthetic devices to be interfaced with cellular signaling pathways and their ability to detect disease biomarkers and link this detection to regulated gene-expression events.

To examine the extension of our device platform to multi-input processing, we constructed devices containing combinations of the WT and mutant MS2 aptamers in positions 3 and 10 (Fig. 3A). Devices containing the WT aptamer in either position displayed significant increases in gene expression ($P < 0.01$) and exon exclusion in the presence of MS2-DsRed compared with that in the absence of ligand (Fig. 3B, fig. S3A, and table S5). A device with aptamers in both positions showed a ~30 to 45% increase in gene expression and exon exclusion compared with that of the single-aptamer devices. We also built multi-input devices to detect heterologous MS2-DsRed and endogenous NF- κ B p50 (Fig. 3C). We inserted the WT and mutant p50(1) and MS2 aptamers into sites 3 and 10, respectively. TNF- α stimulation led to a decrease in gene expression and exon exclusion, whereas expression of MS2-DsRed led to a significant increase in gene expression ($P < 0.05$) and exon exclusion from this device (Fig. 3D, fig. S3, B and C, and table S6). The device response in the presence of both ligands was greater than the sum of the individual ligand output signals, suggesting that the combined inputs have a synergistic effect on the output signal. These studies indicate that our device platform can support combinatorial regulation of gene expression in response to multiple protein inputs.

To examine whether our protein-responsive RNA devices can be used to regulate cell-fate decisions, we developed devices that integrated across two therapeutic inputs—increased signaling via a disease-associated pathway and the presence of an exogenously applied, inactive “pro-drug”—to trigger targeted cell death (Fig. 4A). We constructed β -catenin- and NF- κ B-responsive devices that trigger apoptosis by replacing the output module with a gene encoding the herpes simplex virus–thymidine kinase (HSV-TK) (Fig. 4, B and C). HSV-TK confers sensitivity to the pro-drug ganciclovir (GCV), which induces apoptosis (28) and has been used in clinical trials to treat tumors (29). Cells stably expressing the

β -cat-6 and p65-3 devices exhibited increased sensitivity to GCV under pathway stimulation (fig. S4, A and B) and a cell survival of ~20% at 100 μ g/ml GCV ($P < 0.01$) (Fig. 4D), similar to that of cells overexpressing HSV-TK (fig. S4C). Cells expressing the devices in the absence of pathway stimulation and mutant devices under pathway stimulation displayed no observable phenotypic effects and survival rates between 60 to 90% at 100 μ g/ml GCV. We demonstrated the modularity of the output function of the device platform by replacing the output module with the pro-apoptotic gene *Puma* (fig. S5).

These results demonstrate that our RNA devices can effectively rewire signaling through disease-associated pathways to trigger apoptosis with the use of clinically relevant genetic systems. There was a slight reduction in survival in cells expressing our devices in the absence of pathway stimulation, which may reflect effects of high GCV concentrations on cell viability (fig. S4D) (30) and basal expression of HSV-TK from our devices. Possible therapeutic utility and safety of these targeted-cell-death devices is supported by effective cell-killing efficacy in the presence of both inputs and minimal background activity in the absence of one or both inputs. Our results demonstrate that synthetic RNA controllers with moderate gene-regulatory activities (~two- to fourfold) can achieve substantial alterations in downstream functional behaviors through their coupling to potent genetic targets and effects that are amplified through associated cellular pathways.

Our system demonstrates that heterologous and endogenous proteins not associated with splicing regulation can be directed to alter splicing patterns through synthetic protein-binding sequences. In contrast to protein-based transcriptional control systems, RNA-based systems present advantages in enabling response to proteins other than transcription factors, direct tailoring of input/output processing functions without device redesign, extension to combinatorial processing, and practical implementation in clinical applications (9, 31). The extension of our framework to the processing of multiple protein inputs can be used to engineer RNA-based devices with sophisticated information processing activities and to design and build complex regulatory networks to interrogate and program cellular function.

References and Notes

- W. Weber, M. Fussenegger, *Chem. Biol.* **16**, 287 (2009).
- A. S. Khalil, J. J. Collins, *Nat. Rev. Genet.* **11**, 367 (2010).
- C. J. Bashor, N. C. Helman, S. Yan, W. A. Lim, *Science* **319**, 1539 (2008).

- M. M. Babu, N. M. Luscombe, L. Aravind, M. Gerstein, S. A. Teichmann, *Curr. Opin. Struct. Biol.* **14**, 283 (2004).
- J. M. Vaquerizas, S. K. Kummerfeld, S. A. Teichmann, N. M. Luscombe, *Nat. Rev. Genet.* **10**, 252 (2009).
- K. H. Link, R. R. Breaker, *Gene Ther.* **16**, 1189 (2009).
- E. A. Davidson, A. D. Ellington, *Nat. Chem. Biol.* **3**, 23 (2007).
- B. Suess, J. E. Weigand, *RNA Biol.* **5**, 24 (2008).
- M. N. Win, J. C. Liang, C. D. Smolke, *Chem. Biol.* **16**, 298 (2009).
- F. J. Isaacs *et al.*, *Nat. Biotechnol.* **22**, 841 (2004).
- B. D. Brown *et al.*, *Nat. Biotechnol.* **25**, 1457 (2007).
- K. Rinaudo *et al.*, *Nat. Biotechnol.* **25**, 795 (2007).
- J. Villemain, I. Dion, S. A. Elela, B. Chabot, *J. Biol. Chem.* **278**, 50031 (2003).
- A. D. Ellington, J. W. Szostak, *Nature* **346**, 818 (1990).
- C. Tuerk, L. Gold, *Science* **249**, 505 (1990).
- Materials and methods are available as supporting material on Science Online.
- A. J. Matlin, F. Clark, C. W. Smith, *Nat. Rev. Mol. Cell Biol.* **6**, 386 (2005).
- J. Carey, P. T. Lowary, O. C. Uhlenbeck, *Biochemistry* **22**, 4723 (1983).
- S. J. Culler, K. G. Hoff, R. B. Voelker, J. A. Berglund, C. D. Smolke, *Nucleic Acids Res.* **38**, 5152 (2010).
- L. Cartegni, M. L. Hastings, J. A. Calarco, E. de Stanchina, A. R. Krainer, *Am. J. Hum. Genet.* **78**, 63 (2006).
- D. S. Kim, V. Gusti, K. J. Dery, R. K. Gaur, *BMC Mol. Biol.* **9**, 23 (2008).
- G. M. Arndt *et al.*, *BMC Cancer* **9**, 374 (2009).
- J. Mi *et al.*, *Nucleic Acids Res.* **34**, 3577 (2006).
- R. Chan *et al.*, *Nucleic Acids Res.* **34**, e36 (2006).
- S. E. Wurster, L. J. Maher 3rd, *RNA* **14**, 1037 (2008).
- C. E. Hellweg, A. Arenz, S. Bogner, C. Schmitz, C. Baumstark-Khan, *Ann. N.Y. Acad. Sci.* **1091**, 191 (2006).
- H. K. Lee *et al.*, *Cancer Res.* **67**, 9315 (2007).
- C. Beltinger *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **96**, 8699 (1999).
- Y. Lifang, T. Min, A. Midan, C. Ya, *J. Exp. Clin. Cancer Res.* **27**, 42 (2008).
- Y. Song, B. Kong, D. Ma, X. Qu, S. Jiang, *Int. J. Gynecol. Cancer* **16**, 156 (2006).
- Y. Y. Chen, M. C. Jensen, C. D. Smolke, *Proc. Natl. Acad. Sci. U.S.A.* **107**, 8531 (2010).
- We thank K. Hertel for providing the pHis-BIVT-MS2-Rsp55 construct, M. Jensen for providing pCD19t-Tk-T2A-IL15op_ePHIV7, L. J. Maher for providing pGAD24, and B. Stewart for laboratory assistance. This work was supported by the Caltech Joseph Jacobs Institute for Molecular Engineering for Medicine (grant to C.D.S.), the NIH (fellowship to K.G.H., grant to C.D.S.), the U.S. Department of Defense (grant to C.D.S.), the Alfred P. Sloan Foundation (fellowship to C.D.S.), and the Bill and Melinda Gates Foundation (grant to C.D.S.). The authors have filed for a patent related to this work.

Supporting Online Material

www.sciencemag.org/cgi/content/full/330/6008/1251/DC1
Materials and Methods
SOM Text
Figs. S1 to S9
Tables S1 to S10
References

11 May 2010; accepted 6 October 2010
10.1126/science.1192128

NEW PRODUCTS



FLAME PHOTOMETER

The model 360 Flame Photometer responds to the current demand that five filters come standard in a low-cost instrument. This instrument brings unrivalled precision, ergonomic design, and ease of maintenance to industrial flame photometers. The photometer is fundamentally more stable, less noisy, and has lower limits of detection than would be expected in such a cost-effective instrument. Ease of operation is ensured by the ergonomic design, which uniquely places all the controls, the pressure gauge, air regulator, and sample introduction on the front of the instrument. The large unobstructed sample work area features an innovative spill containment tray which is easy to remove and clean. LEDs on the front panel indicate the filter selected—sodium, potassium, lithium, calcium, or barium—and the flame status. Applications include the analysis of sodium in food, drinking water, waste water, and cement manufacture. The 360 is also an ideal choice for the education sector. The instrument has a conveniently small footprint of 20 x 30 cm and provides exceptional ease-of-maintenance.

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Science Careers

From the journal *Science* 

POSITIONS OPEN

OPEN-RANK, TENURE-TRACK FACULTY POSITION IN BACTERIOLOGY

Department of Microbiology, Immunology, and
Pathology
Colorado State University
Fort Collins, Colorado

The Department of MIP ([website: http://www.cvms.colostate.edu/mip/](http://www.cvms.colostate.edu/mip/)) is seeking to fill an open rank tenure-track position in Bacteriology at the **ASSISTANT, ASSOCIATE, or FULL PROFESSOR** level. This is an international search for a scientist who will join a dynamic department working on fundamental, clinical and bio-security aspects of a range of bacteria ([website: http://www.cvms.colostate.edu/ns/departments/mip/](http://www.cvms.colostate.edu/ns/departments/mip/)) with particular strengths in: mycobacteriology, notably in tuberculosis and leprosy; *Pseudomonas aeruginosa*; *Burkholderia pseudomallei*; and *Francisella tularensis*. Studies of these pathogens are closely affiliated with the Mycobacteria Research Laboratories, the Rocky Mountain Regional Center of Excellence for Biodefense and Emerging Infectious Diseases Research, and the Rocky Mountain Regional Biocontainment Laboratory. The new hire will reinforce existing disciplines in the Department or steer the Department into new areas of research. Candidates with the demonstrated promise or a history of establishing and maintaining a successful research program are being sought. We are particularly interested in individuals with research programs in drug development, resistance, and pathogenesis, but applicants in all areas of bacteriological research will be considered.

The successful candidate will participate in the MIP undergraduate, graduate, and professional teaching programs as appropriate, and will have multiple opportunities to collaborate and assume a leadership role in infectious disease research programs at Colorado State University (CSU). The exceptional research and teaching opportunities at CSU are enriched by the highly interactive and collaborative environment with world recognized Infectious Diseases programs and scientists on campus and the Centers for Disease Control laboratories on the CSU Foothills Campus.

Candidates must have the Ph.D., D.V.M., or M.D. degree and an established record of scholarly activity in bacteriology. Salary and startup package will be commensurate with experience and are negotiable.

More information concerning the position is available at [website: http://www.cvms.colostate.edu/mip/Jobs/bacteriologist.aspx](http://www.cvms.colostate.edu/mip/Jobs/bacteriologist.aspx).

Applications will be accepted until the position is filled. However, evaluations of applications will begin by February 14, 2011. Candidates should electronically submit: a letter of application, curriculum vitae, and the names, mailing, and e-mail addresses of at least three references to:

Herbert P. Schweizer, Ph.D.

Chair of the Bacteriology Search Committee

E-mail: herbert.schweizer@colostate.edu

CSU is an Equal Opportunity/Affirmative Action Employer and conducts background checks on all final candidates.

POSTDOCTORAL ASSOCIATE

The Lillehei Heart Institute is a premier institute at the University of Minnesota in Minneapolis with state-of-the-art technologies and core facilities focused on the molecular regulation of myogenesis, stem cell, and regenerative biology. We are accepting applications for highly motivated postdoctoral associates to work on NIH-funded research pertaining to the role of transcription factor mediated networks to direct the fate of stem and iPS cells to a mesodermal fate (i.e. cardiac, endothelial, skeletal muscle). Ph.D. and expertise with molecular biological-biochemistry techniques as well as the use of transgenesis and knockout technologies will be required for this position. Interested applicants should apply online to: **Daniel J. Garry, M.D.-Ph.D., Director of Lillehei Heart Institute, University of Minnesota** at [website: http://employment.umn.edu](http://employment.umn.edu) (reference job search #166342) and include curriculum vitae, statement of interest, and names of three references. *Candidates must be able to demonstrate authorization to work in the United States at the University of Minnesota by the start date. The University Of Minnesota is an Affirmative Action/Equal Opportunity Employer.*

POSITIONS OPEN



PIADC BIOSAFETY OFFICER

This is an exciting opportunity for an experienced Biological Safety Officer to work at the U.S. Department of Homeland Security Plum Island Animal Disease Center (PIADC). PIADC is the Nation's number one laboratory responsible for protecting livestock from foreign animal diseases. Scientists from both DHS and USDA work at this facility where they conduct basic and applied research to better understand foreign animal diseases, develop vaccines, and maintain and improve the Nation's foreign animal disease diagnostic capabilities.

We are seeking an energetic individual with proven experience in coordinating and guiding biological safety, radiation safety, and animal care programs to serve as a recognized authority regarding biological safety and biological containment in support of PIADC's scientific mission.

Candidates must be U.S. citizens and hold a degree in biological sciences, agriculture, natural resource management, chemistry, or related disciplines appropriate to the position, or a combination of education and experience. Additional requirements are outlined on the website identified below. The selected applicant must successfully complete security background checks, including for Select Agents, and will be subject to random and applicant drug testing.

To apply to the vacancy announcement and see a copy of the qualifications, please go to [website: http://www.jobview.usajobs.gov](http://www.jobview.usajobs.gov) and search for **announcement number DSHQ11-398615-ST**. Applicants must address and respond specifically to the Knowledge, Skills, and Abilities ("KSAs") criteria to be evaluated and considered for the position. This position has a salary range of \$77,585-\$119,935 per annum (GS-0401-12/13). Relocation costs may be paid. *DHS is an Equal Opportunity Employer.*

POSTDOCTORAL RESEARCH ASSOCIATE POSITION

A postdoctoral research position is available to perform research on Orthopoxviruses and protein based assay development and immunology. The Centers for Disease Control and Prevention, Poxvirus and Rabies Branch (NCEZID/DHCPP/PRB) has begun development of panels of hybridomas previously generated against Orthopoxviruses including Variola, and will initiate screening of these clones to define virus specific monoclonal antibodies for use in immunological capture assays (e.g., ELISAs, LIAs). The research program has available preexisting resources and collections of Variola and related near-neighbor viral strains to develop, screen, and characterize hybridomas to identify monoclonal antibodies with specific reactivity to Variola and other Orthopoxviruses (PAN) of interest for diagnostic and detection potential. Specific tasks of the position will include; performing hybridoma screening to define clones with reactivity to Orthopoxviruses (PAN) and also those specific for Variola, the development of proteome arrays using Orthopoxvirus genome cloning, and ongoing transcriptional expression of Orthopoxvirus proteins in vitro for sustained array production, pilot capture assays to define detection capacities of monoclonal antibodies and refine ELISA based formats for development of Surveillance and Detection assays, assess select monoclonal antibodies for protein specific reactivity using proteome microarrays and isolation of subsequently identified virus clones, and develop rapid assay formats using identified monoclonal antibodies for surveillance and detection. Reagents of interest will be used in biological studies of Orthopoxviruses. Previous experience in protein microarrays and antibody assays and assay development is desirable. Some experience in molecular biology and clonal expression is also desirable. Please submit an application directly to **e-mails: kkarem@cdc.gov or volson@cdc.gov** that includes current curriculum vitae, list of publications and awards, and contact information for five references.



University of Bern
Faculty of Science

Applications are invited for a full professorship in
Fundamental Astronomy/Space Geodesy
including the Directorate of the Astronomical Institute,

opening 1 August 2011, at the Astronomical Institute, University of Bern, Switzerland (<http://www.aiub.unibe.ch>). Candidates should have a strong research record in fundamental astronomy, e.g., **space geodesy** and **astrodynamics** in the broadest sense. Current research activities of the institute include space geodesy using GNSS and satellite laser ranging, gravity field determination and optical surveys for natural and artificial bodies in the solar system. Future research might be extended towards the study of the orbital and rotational motion of planets including their natural and artificial satellites, and to other planetary system bodies such as asteroids, and comets. The institute develops and operates the Zimmerwald Observatory. The successful candidate is expected to direct the institute's research activities, to attract external funding, and to actively participate in the teaching and supervision of Bachelor and Master students, as well as Ph.D. students.

The University of Bern particularly encourages women to apply for this position.

Applications including a curriculum vitae, a publication list, copies of the five most important publications, a brief outline of past and future research, and a list of external funds raised, should be sent either as a hard copy to Prof. S. Decurtins, Dean of the Faculty of Science, University of Bern, Sidlerstrasse 5. CH-3012 Bern, Switzerland, or as a single PDF-file via e-mail to: dekan@natdek.unibe.ch, by **31 January 2011**.



PROGRAM LEADER International Institute for Applied Systems Analysis

The International Institute for Applied Systems Analysis (IIASA, www.iiasa.ac.at), located near Vienna, Austria, is seeking a Program Leader for its Ecosystems Services and Management Program beginning spring 2011. The successful candidate will work closely with the Food and Water Area Leader and other Program Leaders to head a team of about 30 researchers with interdisciplinary backgrounds ranging from forestry and agriculture, geography and land use, to economics and mathematics. In addition to managing an annual budget of approx. EUR 3 million and promoting the development of externally funded research, the Program Leader will develop integrated methodologies, establish new applications and search for policy-relevant solutions to global and regional problems in the area of food and water security, use and ecosystems management as well as developing international and in-house cooperation and networks on land use, ecosystem services and food and water systems, plus other linked cross-cutting activities.

Candidates should combine a vision for IIASA's Ecosystems Services and Management Program with scientific excellence, management and diplomatic skills, and broad experience in interdisciplinary research and policy applications in the international arena.

IIASA is nongovernmental, sponsored by an international consortium of 17 National Member Organizations. Applicants should have excellent written and spoken English, IIASA's working language. The Institute's management and staff alike are committed to a working environment that promotes equality, diversity, and tolerance.

An initial contract of 3 years will be offered with the possibility of extension. IIASA offers a competitive compensation and benefits package and salaries are exempt from taxation in Austria. Review of applications will begin immediately. To apply, e-mail a cover letter, CV, contact details of 3 work-related reference givers and copies of five key publications to:

Ms. Alia Harrison, Recruitment Officer
International Institute for Applied Systems Analysis (IIASA)
E-mail: harrison@iiasa.ac.at

Neuroscience postdoc programme

Linköping University is one of Sweden's six large universities, currently enrolling 26,500 students. The university recently launched a Centre for Neurobiology, involving some 19 independent research groups from two faculties: the Faculty of Medicine and the Faculty of Science and Engineering.

The Centre for Neurobiology is now seeking Postdoctoral Fellows (2+2 years) within several neuroscience areas: Addiction, Behavior, Electrophysiology and Circuits, Imaging and navigation, Neurodegeneration, Neuroendocrinology, Neurodevelopment and Pain.

For more details regarding the Centre, the different research labs involved in the program, and to submit a letter-of-intent please go to: <http://www.hu.liu.se/neuro?!=en>. For information regarding the university and the region, please go to: <http://www.hu.liu.se/?l=en>, <http://www.liu.se/?l=en>, <http://www.twincities.se/>



Linköping University

FEATURED EMPLOYER



Faculty Position in Microbial Ecology at Arizona State University - Tempe, AZ

The School of Life Sciences and The Biodesign Institute at Arizona State University (ASU) invite applications for a tenure-track position at the rank of Assistant Professor in the field of Microbial Ecology. We seek creative individuals who study basic aspects of microbial interactions with the biotic or abiotic environment using cutting-edge techniques, and who are motivated to develop an academic career at ASU. The successful candidate will be expected to develop an innovative, extramurally funded, independent research program, fulfill teaching requirements at both the undergraduate and graduate levels, mentor undergraduate, graduate and postdoctoral students and have a commitment to outreach and service at levels within and outside the University community. Preference will be given to candidates whose research complements existing areas of expertise in the School (www.sols.asu.edu) and The Biodesign Institute (www.biodesign.asu.edu). A competitive startup package and a teaching load compatible with high research productivity will be provided.

Candidates must have a doctoral degree in an appropriate field, and minimally one year of relevant postdoctoral experience at the time of appointment. Demonstrated teaching and research excellence is preferred.

To apply, send a cover letter, your curriculum vitae, three representative publications, separate statements of future research plans and teaching philosophy and interests, and arrange for three letters of reference to be sent to **Ferran Garcia-Pichel, Chair, Microbial Ecology Faculty Search Committee, School of Life Sciences, PO Box 874501, Tempe, AZ 85287-4501**. Electronic applications sent as PDF files to solsfacultysearch@asu.edu are preferred. The initial closing date for receipt of applications is **January 17, 2011**; applications will be reviewed weekly thereafter until the search is closed. A background check is required for employment. For additional information on this position and the School of Life Sciences, please visit <http://sols.asu.edu/jobs>.

Arizona State University is an Affirmative Action, Equal Opportunity Employer committed to excellence through diversity. Women and minorities are encouraged to apply.



Faculty Position in Metabolic and Vascular Biology at Arizona State University -Tempe, AZ

The School of Life Sciences and The Biodesign Institute at Arizona State University (ASU) invite applications for a tenure-track position at the rank of Assistant Professor in the area of Metabolic and Vascular Biology. We seek individuals who will join in the mission to unravel the mechanisms underlying insulin resistance, obesity, vascular disease, and type 2 diabetes mellitus. This position is part of a major expansion in the life sciences at Arizona State University, and tied to the Mayo Clinic/ASU Center for Metabolic and Vascular Biology at ASU and the Mayo Clinic Scottsdale.

The successful candidate will be expected to develop an innovative, extramurally funded, independent research program, fulfill teaching requirements at both the undergraduate and graduate levels and have a commitment to outreach and service at levels within and outside the University community. The successful candidate will be expected to mentor undergraduate, graduate and postdoctoral students and interact in the multidisciplinary Center for Metabolic and Vascular Biology. A competitive startup package and teaching load compatible with high research productivity will be provided.

Candidates must have a doctoral degree in a related discipline at the time of appointment, and at least two years of relevant postdoctoral research experience. Preferred areas of expertise are functional genomics or proteomics, phenotyping and characterizing mouse models, and generation and use of such models to address questions of altered gene expression or insulin signaling in insulin resistance. Research areas could include the role of inflammatory response, lipids, or mitochondrial dysfunction in insulin resistance. Demonstrated teaching and research excellence is preferred.

To apply, send a cover letter, your curriculum vitae, three representative publications, separate statements of future research plans and teaching philosophy and interests, and arrange for three letters of reference to be sent to **Lawrence Mandarino, Chair, Metabolic Biology Search Committee, School of Life Sciences, PO Box 874501, Tempe, AZ 85287-4501**. Electronic applications sent as PDF files to solsfacultysearch@asu.edu are preferred. The initial closing date for receipt of applications is **January 17, 2011**; applications will be reviewed weekly thereafter until the search is closed. A background check is required for employment. For additional information on this position and the School of Life Sciences, please visit <http://sols.asu.edu/jobs>.

Arizona State University is an Affirmative Action, Equal Opportunity Employer committed to excellence through diversity. Women and minorities are encouraged to apply.



Faculty Position in Immunology at Arizona State University -Tempe, AZ

The School of Life Sciences and The Biodesign Institute at Arizona State University invite applications for a tenure-track position at the rank of Assistant Professor in the area of Immunology. We seek imaginative, creative individuals with motivation to provide basic understanding of basic immunology and the immunological mechanisms by which animal hosts deal with pathogens. The successful candidate will be expected to develop an innovative, extramurally funded, independent research program, fulfill teaching requirements at both the undergraduate and graduate levels and have a commitment to outreach and service at levels within and outside the University community. The successful candidate will be expected to mentor undergraduate, graduate and postdoctoral students, and interact in the multidisciplinary consortium of faculty in the Center for Infectious Diseases and Vaccinology (<http://cidv.biodesign.asu.edu>) in The Biodesign Institute. There are no preferences as to the aspects (mucosal, systemic or cellular) of the immune system selected for study. However, preference will be given to applicants who have interests that complement expertise of existing faculty and who will expand our overall research and instructional capabilities. A competitive startup package and a teaching load compatible with high research productivity will be provided.

Candidates must have a doctoral degree in an appropriate field at the time of appointment and two or more years of relevant postdoctoral experience. Demonstrated teaching and research excellence is preferred.

To apply, send cover letter, your curriculum vitae, three representative publications, separate statements of future research plans and teaching philosophy and interests, and arrange for three letters of reference to be sent to **Josephine Clark-Curtiss, Co-Chair, Immunology Faculty Search Committee, School of Life Sciences, PO Box 874501, Tempe, AZ 85287-4501**. Electronic applications sent as PDF files to solsfacultysearch@asu.edu are preferred. The initial closing date for receipt of applications is **January 17, 2011**; applications will be reviewed weekly thereafter until the search is closed. A background check is required for employment. For additional information on this position and the School of Life Sciences, please visit <http://sols.asu.edu/jobs>.

Arizona State University is an Affirmative Action, Equal Opportunity Employer committed to excellence through diversity. Women and minorities are encouraged to apply.



Faculty Positions in Genomics at Arizona State University - Tempe, AZ

The School of Life Sciences and The Biodesign Institute at Arizona State University invite applications for three tenure-track faculty positions at the level of Assistant Professor in the area of Genomics. We seek outstanding candidates who employ an integrated approach to address fundamental principles in the development, evolution, characterization and diagnosis of health-related disorders. Methods can include theoretical and empirical approaches in population genetics, functional and comparative genomics, bioinformatics, and experimental studies. Successful candidates will be expected to develop an innovative, extramurally funded, independent research program, fulfill teaching requirements at both the undergraduate and graduate levels, mentor undergraduate, graduate and postdoctoral students and have a commitment to outreach and service at levels within and outside the University community. A competitive startup package and teaching load compatible with high research productivity will be provided.

Arizona State University has made a commitment to accelerating the translation of basic discoveries into practical benefits for society through the construction of state of the art research facilities and the recruitment of world-class faculty. Successful candidates would participate in a university-wide health initiative supported by core facilities for functional genomics and next generation sequencing, functional proteomics, high throughput cellular screen, bioinformatics, high performance computing, and imaging. More information on genomic research opportunities at ASU can be found at <http://genomics.asu.edu>.

Candidates must have a Ph.D. (or equivalent) in an appropriate field, and a minimum of 2 years of postdoctoral training is preferred. Demonstrated teaching and research excellence is preferred.

To apply, send cover letter, your curriculum vitae, three representative publications, separate statements of future research plans and teaching philosophy and interests, and arrange for three letters of reference to be sent to **Alan Rawls, Chair, Genomics Faculty Search Committee, School of Life Sciences, PO Box 874501, Tempe, AZ 85287-4501**. Electronic applications sent as PDF files to solsfacultysearch@asu.edu are preferred. The initial closing date for receipt of applications is **January 17, 2011**; applications will be reviewed weekly thereafter until the search is closed. A background check is required for employment. For additional information on this position and the School of Life Sciences, please visit <http://sols.asu.edu/jobs>.

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NIOZ Royal Netherlands Institute for Sea Research is the Dutch national marine and oceanographic institute. It is one of the world's leading marine research centres; research involves studies within the traditional disciplines as well as multidisciplinary studies in physical oceanography, marine chemistry, biogeochemistry, molecular biology, microbiology, ecology and geology. The main division of the institute is located on the island of Texel.

The scientific staff consists of some 140 researchers (PhD students and post docs included) NIOZ employs 160 support staff in laboratories, technical departments and on research vessels. The annual budget of NIOZ is approximately € 30m.

NIOZ participates in numerous national and international scientific collaborations (universities, scientific institutes and scientific organizations). The institute also contributes significantly to marine education in the Netherlands and Europe.



Netherlands Organisation for Scientific Research



V A C A N C Y

■ General Director

NIOZ Royal Netherlands Institute for Sea Research invites applications and nominations for the position of General Director

Job description and requirements

- You will initiate, stimulate and lead the scientific programme of NIOZ at the frontiers of science and technology. You must enjoy an international reputation in the oceanographic or related sciences in combination with first-rate management skills.
- You think strategically, you will be a visionary when it comes to marine and oceanographic research, and you will possess the initiative and diplomatic qualities needed to turn this vision into a coherent and feasible long-term research programme.
- You can operate in a complex and varied national and international network. You have proven capabilities to build, maintain and productively use this network in an effective way. You act as a representative of the institute in general, promoting the institute's interests at policy-making bodies.
- You will initiate and stimulate national and international initiatives to raise funds for research at the institute.
- You are responsible for the dissemination of scientific, marine and oceanographic knowledge for a better understanding and sustainable use of our planet. You have a natural talent for communicating results to the general public.
- You are responsible for the integral management of the institute, including its position as the national Marine Research Facility.
- You will report and be accountable to the Board of NIOZ.

Employment conditions

This is a full-time position, location Texel, the Netherlands. You will be employed by NIOZ. The initial appointment will be for five years, with the possibility of extension for another 5 years. The salary is in accordance with the Dutch regulations on salaries in public management. NIOZ offers good fringe benefits.

Information

For more information about the position of General Director please contact the chair of the Scientific Committee of NIOZ, Prof. Will De Ruijter, +31 623888444 or w.p.m.deruijter@uu.nl. For procedural information please contact Mr. Jos Lensen of the NWO HRM Department, +31 70 344 0685 or j.lensen@nwo.nl.

Application

This post is open to qualified applicants of all nationalities.

A letter of application with a full curriculum vitae (by email as well as by mail) should be sent to HRM@nwo.nl and to NWO HRM Department, P.O. Box 93138, 2509 AC The Hague, Netherlands. Initial consideration will be given to applications received by December 20, 2010.

For more information see www.nioz.nl and www.nwo.nl.



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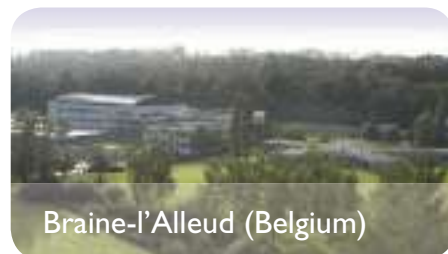
Post-Doctoral Fellowship Programme: **UCB seeking world-class post docs to lead pioneering research projects.**

UCB, a global biopharmaceutical leader focusing on central nervous system and immunological disorders, is currently seeking leading-edge scientists to join our post doctoral programme based at research centres in Belgium and the UK*.

UCB is a patient-centric biopharmaceutical company dedicated to the research, development and commercialization of innovative medicines. Already the leader in epilepsy, UCB is committed to providing novel solutions for people with severe conditions such as Parkinson's disease, rheumatoid arthritis, and restless legs syndrome. Employing more than 9,000 people in over 40 countries, UCB produced revenues of €3.1 billion in 2009. UCB's Research & Development organization has brought medicines such as Zyrtec®, a leading allergy medication, Keppra®, an antiepileptic drug with over 4.1 million patient years' experience, and Cimzia®, the only PEGylated anti-TNF (Tumor Necrosis Factor) for the treatment of autoimmune diseases, to patients around the world.

UCB NewMedicines is UCB's discovery research to clinical proof-of-concept organization charged with building a rich pipeline of differentiated molecules by channeling exquisite science for industry-beating performance. Our research centres are home to a wealth of internal skills and experience in our chosen therapeutic areas, allied to unique and proprietary technology platforms. Our highly effective networks with leading academic and industrial partners ensure access to novel technologies, targets and collaborative services. Our intellectual and scientific capability is evidenced not only by our record of proven delivery but also by the impressive publication record of our scientists in leading journals – for a list of some of UCB's recent publications please visit www.ucb.com/Research&Development.

Our post doctoral scientists will be at the forefront of UCB's cutting edge scientific activities, mentored by UCB's scientific experts on specific projects and regularly interacting with UCB's scientific leadership team. They will be offered extensive opportunities to participate in UCB's broader scientific discussions and debates, actively encouraged to regularly present their research and achieve external publication in eminent scientific journals.



Braine-l'Alleud (Belgium)



Slough (UK)



If you want to develop your career with a global leader and contribute to our outstanding record of achievement please view the post-doctoral opportunities available through our 2011 programme. Further details on the specific projects for which we are recruiting and details of how to apply are available at www.ucb.com/Research&Development.

*Brussels (BE) and Slough (UK)



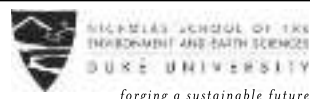
The Department of Medicine/Division of Infectious Diseases and Geographic Medicine at Stanford University is recruiting for three positions at the Assistant Professor or Associate Professor levels to contribute to the research, clinical, and educational activities of the Division. The faculty positions are in the University Tenure Line. Candidates with research programs in all areas of Infectious Diseases will be considered. Those with research programs in Immunology or Global Health are especially encouraged to apply.

Candidates should be board certified in Infectious Diseases. Recruits will be expected to develop a laboratory-based investigative program at Stanford. We anticipate that for each individual's effort, 70% - 80% will focus on research with 20% - 30% distributed between clinical and teaching activities. Independent grant funding, publication record, and programmatic synergy with members of the Division will be considered. The Division of Infectious Diseases and Geographic Medicine benefits from an outstanding scientific and clinical environment at Stanford, including collaborations with colleagues in Microbiology and Immunology, Genetics, Bioengineering, Stanford Institute for Immunity, Transplantation, and Infection, the Stanford Cancer Center and the Woods Institute for the Environment.

The overriding requirement for faculty appointment, reappointment and promotion within the UTL must be distinguished performance, or (in the case of junior faculty) the promise of distinguished performance. There should be a major commitment to research and teaching. There must be outstanding accomplishments in research and excellent overall performance in teaching, as well as in clinical care and institutional service appropriate to the programmatic need the individual is expected to fulfill.

Candidates should submit a detailed letter of interest, curriculum vitae, and three letters of recommendation to: **David Relman M.D., Chair of Search Committee, Stanford University School of Medicine, 300 Pasteur Drive, MC: 5107, Stanford CA 94305-5107.** Applications received by **February 15, 2011** will be given full consideration; applications will be considered on a rolling basis until the positions are filled.

Stanford University is an Equal Opportunity Employer and is committed to increasing the diversity of its faculty. It welcomes nominations of and application from women and members of minority groups, as well as others who would bring additional dimensions to the university's research, teaching and clinical missions.



Hire in the Nicholas School of the Environment in Tropical Ecology November 2010

The Nicholas School of the Environment (NSOE) at Duke University will make a tenure-track appointment for an Assistant Professor in tropical ecology. This position builds on Duke's strengths in ecological, environmental, and biological sciences and seeks to attract an outstanding faculty member who will engage in and facilitate multidisciplinary interactions across the NSOE and other units on campus such as Biology, Evolutionary Anthropology, the Nicholas Institute for Environmental Policy Solutions, the Organization for Tropical Studies, and the Global Health Institute. The successful applicant will carry out research at the interface of tropical ecology, ecosystem function, and the rapid and imposing changes now ongoing across nearly all of the tropics, as well as contribute to the NSOE's curricula at the undergraduate, professional master's, and doctoral levels.

Consideration of applications will begin immediately and continue until the position is filled. Applications should include a full CV, and statements of research and teaching goals (all in one PDF file), and three letters of reference. Applications should be forwarded to **Laura Turcotte (ljturco@duke.edu)**.

The Nicholas School and Duke University are committed to equal opportunity in employment. Applications are strongly encouraged from members of underrepresented populations.

FACULTY POSITIONS Department of Basic Science & Craniofacial Biology COLLEGE OF DENTISTRY

The New York University College of Dentistry (NYUCD) is one of the oldest and largest academic dental institutions in the US. NYUCD is a vital and growing research institution committed to education of the next generation of clinicians and scientists. It is located in Manhattan adjacent to the NYU Medical School and hospital complex. Dr. Nicola C. Partridge is the Chair of Basic Science and Craniofacial Biology. She has recently recruited three tenure track assistant professors working in craniofacial and bone biology and cancer. She is actively recruiting faculty undertaking research in either stem cells, cancer, or bone biology. Two full time, tenure track, positions are available to be filled in the 2011-2012 academic year. Appointments will be tenure track at the Assistant/Associate Professor/Professor rank in the Department of Basic Science & Craniofacial Biology. Candidates are required to have a doctoral degree, currently have research funding, and be ready to implement an independent research program. Excellent start up packages and salary support will be available as well as expanded and renovated laboratory space. NYU has recently merged with the Brooklyn Polytechnic University. Tremendous opportunities for collaboration with engineering and physical scientists are possible.

Responsibilities include: engaging in basic and/or healthcare related research, which should complement established research programs in the college including Bone Biology, Cancer, Tissue Engineering/Stem Cells, and/or Infectious Disease; team teach courses in the basic sciences to students from a range of health care related disciplines; and institutional commitment to the University, College, and Department.

NYUCD has an excellent benefits package. Salary and academic rank will be commensurate with qualifications and experience. Please submit a letter of application, CV, statement of proposed research, and have three letters of recommendation sent to: **Candy S. Petrolle, Department Manager, Department of Basic Science & Craniofacial Biology, New York University College of Dentistry, 345 East 24th Street, New York, NY 10010-4020** or via e-mail at **csp3@nyu.edu**.



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IST AUSTRIA IS LOOKING FOR

Professors and Assistant Professors

IST Austria (Institute of Science and Technology Austria) is a new Institute located on the outskirts of Vienna, dedicated to cutting-edge basic research. The Institute invites applications and nominations for Professors and Assistant Professors in all fields of the natural sciences and related disciplines. Outstanding scientists in the Physical Sciences, Neurosciences, and Mathematics are especially encouraged to apply.

The Institute, established by the Austrian Government, opened its campus in 2009. Its funding is substantial, allowing for over 500 employees and graduate students by 2016. IST Austria is entitled to award PhD degrees and includes an English-language Graduate School. It aims to achieve an international mix of scientists and recruits them solely on the basis of their individual excellence and potential contribution to research. The Institute is recruiting leaders of independent research groups. Professors have indefinite contracts and Assistant Professors have fixed-term contracts for an initial period of five years, with a possible, but not automatic, renewal for two additional years. Before the end of this period, the scientist will be considered for an indefinite appointment as a Professor at IST Austria, the decision being based on merit only (as is the case for a "Tenure-Track Assistant Professor" at US universities).

The selected candidates will receive a competitive salary and a substantial annual research budget, covering operating expenses and the cost of PhD students, postdoctoral fellows, and technical staff. Additional costs of starting a new laboratory, including instruments and infrastructure, will be offered separately. Scientists are also expected to apply for external research grants.

Applications or nominations to: professor@ist.ac.at or assistant.professor@ist.ac.at (depending on position). Applications must include a CV, list of publications, and research plan. Nominations should include an appraisal of the achievements and scientific qualifications of the nominee. IST Austria is committed to Equality and Diversity. In particular female researchers are encouraged to apply. More information: www.ist.ac.at



Modeling and Simulations in Biology Professor

Stony Brook University, home to many highly ranked graduate research programs, is located 60 miles from New York City on Long Island's scenic North Shore. Our 1,100-acre campus is home to 24,000 undergraduate, graduate, and doctoral students and more than 13,500 faculty and staff, including those employed at Stony Brook University Medical Center, Suffolk County's only academic medical center and tertiary care provider. The University is a member of the prestigious Association of American Universities and co-manager of nearby Brookhaven National Laboratory (BNL).

Stony Brook University's Department of Applied Mathematics and Statistics is seeking a modeling and simulations in biology professor. This new tenure-track or tenured faculty position is anticipated for Fall 2011.

Research areas of interest include those in which mathematics, theory, and computation meet biology. For example, systems biology, bionformatics, computational genomics, and computational structural biology.

For a full position description, application procedures, and/or to apply online, visit www.stonybrook.edu/jobs (JOBS Reference #F-6573-10-11).

Stony Brook University/SUNY is an equal opportunity/affirmative action employer.



Assistant/Associate/Full Professor Faculty Position in Infectious Diseases Research Tenure/Tenure Track Faculty, 9-Month Appointment (80% Research and 20% Teaching) Position Number 103761

The Virginia-Maryland College of Veterinary Medicine at the University of Maryland-College Park, MD, invites applications from the qualified individuals for a tenured/tenure-track faculty position in Immunology of Infectious Diseases and Microbial Pathogenesis. This 9-month appointment will be at the Assistant, Associate or Full Professor level, depending on the qualifications of the selected candidate. The Department has an excellent core facility, which includes BSL-2 and BSL-3 suites.

QUALIFICATIONS: A DVM/PhD or PhD degree with relevant postdoctoral training.

RESEARCH: Current focus is on host-pathogen interaction with emphases on virology, immunology, microbial pathogenesis, epidemiology, and public health. The position requires the candidate to focus on host immunity/host-pathogen interactions with importance for human and animal health, zoonosis and public health. The selected candidate will be expected to develop, maintain, and conduct a productive, extramurally funded research program that will strengthen the current research goals of the College. Applicants at the Associate/Full Professor level are expected to have an established, extramurally-funded research program and a strong publication record. The selected candidate will also be expected to develop and maintain active and productive collaborations, both within and outside the College. Excellent opportunities exist for collaboration with federal agencies (USDA, FDA, NIH) and other University departments.

TEACHING: Active participation in the University's Graduate Program will be required, to include mentoring of graduate students and serving on student advisory committees. The selected candidate will be responsible for the development of one graduate course to be included in the current veterinary medicine curriculum. This new course will be developed in conjunction with active participation on the college's current teaching objectives and mission goals. Salary will be commensurate with rank and experience.

TO APPLY: Applications are accepted through <https://jobs.umd.edu> (sort for position number 103761). Required are (1) cover letter, (2) CV, (3) statement of research interests and plans, (4) statement of teaching philosophy and (5) three reference letters. For best consideration, applications should be received by **January 31, 2011**, or until a suitable candidate is identified.

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POSITIONS OPEN

ASSISTANT OR ASSOCIATE PROFESSOR of Science Teacher Education
University of Nebraska-Lincoln

POSITION—The University of Nebraska-Lincoln (UNL) College of Agricultural Sciences and Natural Resources (CASNR) seeks to fill a tenure-track or tenured position as part of a campus-wide initiative to enhance science teacher education and science education outreach. This position will be one of three cluster hires that will be key to the development of a nationally recognized program of excellence promoting interdisciplinary scholarship focused on the recruitment, education, and retention of outstanding secondary science teachers. The NU-Teach initiative is a partnership among the Colleges of Agricultural Sciences and Natural Resources, Arts and Sciences, and Education and Human Sciences. For more information on the departments, colleges, and Centers affiliated with this initiative, see **website: <http://nuteach.unl.edu>**.

The position, to be associated with tenure homes in Agronomy and Horticulture, Biochemistry, Entomology, Food Science and Technology, Plant Pathology, and/or the School of Natural Resources, is anticipated to emphasize research and/or outreach activities related to student learning in science, particularly in relation to recruitment, education, or retention of future science teachers. The anticipated start date for the position is January 2012.

UNL is a land grant institution with a strong commitment to research, teaching, outreach, and service. UNL is a Carnegie Foundation "high research activity" university and an AAU member.

QUALIFICATIONS—Successful candidate must have a Ph.D. or equivalent in a field of life, agricultural, or natural resources sciences and/or education. Preference will be given to applicants with a record of scholarly activity, demonstrated potential for developing an externally funded research program, and expertise in the teaching of science content or pedagogy to all students.

DUTIES AND RESPONSIBILITIES—Teaching duties will include graduate and undergraduate courses appropriate for the home department, including courses that promote recruitment, training, and/or retention of science teachers. The successful candidate will develop a nationally recognized and externally funded research program, and work with faculty across the entire campus to move UNL to a position of national leadership in science education.

RANK/SALARY/BENEFITS—Assistant or Associate Professor, tenure-track or tenured. Salary is competitive and commensurate with qualifications and experience. UNL offers a benefits package that makes available: group life, health, disability insurance and family coverage programs; TIAA/CREF and/or Fidelity Investment Fund retirement plans; and dependent tuition remission.

APPLICATIONS—To be considered for this position, go to **website: <http://employment.unl.edu>**, search for **requisition number 100765** and complete the faculty academic administrative information form. Applicants should attach curriculum vitae; a statement of research and teaching interests (other document); contact information for three references; and a personal statement, which should also identify a potential CASNR home department (letter of application). Candidates should also arrange for three letters of reference to be submitted electronically to **Linda Arnold (e-mail: larnold1@unl.edu)**. Review of applications will begin January 21, 2011, and continue until the position has been filled or the search is closed.

The University of Nebraska has an active National Science Foundation ADVANCE gender equity program and is committed to a pluralistic campus community through affirmative action, equal opportunity, work-life balance, and dual careers.

POSITIONS OPEN

FACULTY POSITION IN NEUROSCIENCE
Department of Physiology & Biophysics And
Washington National Primate Research Center
University of Washington

The Department of Physiology & Biophysics at the University of Washington and the Washington National Primate Research Center announce a search for a full-time faculty member. Appointment at the **ASSISTANT PROFESSOR** level tenure-track is preferred but **ASSOCIATE PROFESSOR** will also be considered, if appropriate. University of Washington faculty engages in teaching, research, and service. We seek an individual (Ph.D. and/or M.D.) with research interests and demonstrated scholarly achievements in the field of primate neuroscience. The new faculty member will be expected to establish a vigorous research program and participate in teaching of graduate and professional students. For information about the Department, see our **website: <http://depts.washington.edu/pbiopage>**. The new faculty member will also be appointed Core Staff Scientist in the Washington National Primate Research Center.

Application packages should be submitted electronically to **e-mail: pbsearch@u.washington.edu**. Applicants should submit curriculum vitae and a description of research accomplishments and plans and have three letters of reference sent to: **Neuroscience Search Committee, Department of Physiology & Biophysics, Box 357290, University of Washington, Seattle, WA 98195-7290**. Review of applications will begin on December 1, 2010, and continue until the position is filled.

The University of Washington is building a culturally diverse faculty and strongly encourages applications from women and minority candidates. The University of Washington is an Affirmative Action/Equal Opportunity Employer.

ASSISTANT PROFESSOR of BIOCHEMISTRY
Western Michigan University
Department of Chemistry

The Department of Chemistry at Western Michigan University has an available tenure-track faculty position at the rank of Assistant Professor with specialization in Biochemistry that will begin in August 2011 pending budgetary approval. Successful candidates are required to have a Ph.D. degree in Chemistry, good oral and written communication skills, and need to demonstrate a potential for excellence in teaching and extramurally funded research. Candidates will be expected to teach undergraduate, Master's, and Doctoral students. Other duties will include advising graduate students and serving on department, college, and university committees. Modern laboratory facilities as well as a generous startup package for qualified applicants can be anticipated. Qualified candidates should apply online at **website: <http://www.wmich.edu/hr/careers-at-WMU.html>** (click on "Search Job Postings"). Applications should include a cover letter, curriculum vitae, research plan, transcript, teaching philosophy, and three letters of recommendation. Review of applications will begin December 1 and will continue until the position is filled. For further information **e-mail: chem-search@wmich.edu** or visit **website: <http://www.wmich.edu/chem/biochem/>**.

Western Michigan University is a dynamic student-centered research university with an enrollment of 25,000 and is located in Kalamazoo, Michigan, midway between Chicago and Detroit. The Carnegie Foundation for the Advancement of Teaching has placed WMU among the 76 public institutions in the nation designated as research universities with high research activity. *Western Michigan University is an Equal Opportunity Employer consistent with applicable federal and state law. All qualified applicants are encouraged to apply.*

POSITIONS OPEN

FACULTY POSITION IN AQUATIC SCIENCE

The Center for Environmental Studies at Virginia Commonwealth University (VCU, **website: <http://www.vcu.edu/cesweb>**) invites applications for an aquatic scientist whose research integrates fieldwork, modeling, and/or geospatial analysis (e.g. GIS, remote sensing) to understand ecological and environmental processes of large river systems across broad spatial scales. The successful candidate will be expected to develop an active, externally funded research program focused on rivers, direct graduate students through the doctoral level, complement our existing strengths in areas such as restoration ecology, biogeochemistry, and river food web analysis, and contribute to the mission of VCU's Rice Center (**website: <http://www.vcu.edu/rice>**). Teaching responsibilities will include undergraduate and graduate courses in the Environmental Studies curriculum. This is a nine-month, tenure-track position at the rank of **ASSISTANT PROFESSOR**; exceptional candidates at senior levels are also encouraged to apply. Postdoctoral experience is expected and demonstrated evidence of excellence in scholarship is required. Candidates must have demonstrated experience working in and fostering a diverse faculty, staff, and student environment or commitment to do so as a faculty member at VCU. Competitive startup funds are available.

Submit curriculum vitae, statement of research and teaching interest, and contact information for three references by January 15, 2011 to: **Pamela Mason, Center for Environmental Studies, 1000 W. Cary Street, Box 843050, Virginia Commonwealth University, Richmond, VA 23284-3050**.

Virginia Commonwealth University is an Equal Opportunity/Affirmative Action Institution. Women, minorities, and persons with disabilities are encouraged to apply.

POSTDOCTORAL POSITION
Department of Human Genetics
Emory University School of Medicine

Postdoctoral position open in the laboratory of **Dr. Stephen Warren** for a talented, creative, and scientifically adventurous individual with documented research accomplishments and bench skills. Our work covers all aspects of Fragile X syndrome, a common inherited form of cognitive deficiency and autism (see **website: <http://genetics.emory.edu>**). We operate as a dynamic, fast-paced team focused upon discovery and seek motivated applicants with a Ph.D. and/or M.D. degree with training in neurobiology, molecular biology, and/or genetics. Experimental skills in neuronal cell culture, protein translation, histone modification, and/or iPSC cell culture would be useful. Send curriculum vitae and the contact information for three references to **e-mail: christi.bell@emory.edu**. *Emory University is an Equal Opportunity/Affirmative Action Employer.*

A **POSTDOCTORAL POSITION** is available at the University of Maryland School of Medicine to model inherited lipid storage diseases using iPSC cell technology. Our laboratory is using patient-derived reprogrammed cells to elucidate the molecular mechanisms leading to abnormal cell function and to develop stem cell-based therapy. The candidate should have a Ph.D., experience in Cell/Molecular Biology, excellent oral and written communication skills, and be able to work independently. Experience in hES/iPSC technology and hematopoietic/neuronal development is desirable. Salary is commensurate with experience. To apply, send curriculum vitae and contact information for three references to **Dr. Ricardo A. Feldman** at **e-mail: rfeldman@umaryland.edu**.

PETER BUCK FELLOWSHIPS—Smithsonian National Museum of Natural History

The Smithsonian National Museum of Natural History (NMNH) announces the Peter Buck Fellowship Program, which will offer **POSTDOCTORAL** and **GRADUATE FELLOWSHIPS** to those wishing to work with advisors at NMNH. Peter Buck Postdoctoral Fellowships are two to three years in duration, Graduate Fellowships one to two years. Information for Postdoctoral and Graduate applicants is given at **website: <http://www.si.edu/ofg/>**. The next application deadline will be January 15, 2011. *Peter Buck Fellowships are open to citizens of any country.*

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MEETINGS

US-EC Course in Environmental Biotechnology 'Microbial Catalysts for the Environment' July 9-23, 2011 in Lausanne, Switzerland

The University of Lausanne will be the site for the 2011 International Course on Environmental Biotechnology, under the auspices of the EC-US Working Group on Biotechnology for the Environment. This course will be built on the success of the three previous courses in training early career scientists in the state-of-the-art theoretical and practical aspects of microbial environmental biotechnology.

The central topic of the course is 'Microbial Catalysts for the Environment', the concept to identify and select microbial strains suitable for environmental application such as bioremediation or bioreporting. The course program consists of two weeks of hands-on experimental work with two daily lectures on closely related topics in environmental microbiology or biotechnology. Course experiments and techniques will include on site catabolic chip analysis, total community analysis, bioaugmentation and catalyst survival, transcriptomics and/or quantitative real-time PCR, plus bioreporter and bioavailability analysis. Instructors for the course will be from the international European Framework research consortium BACSIN, with special lectures by professors from both US and EC.

The course is limited to 24 students at the level of studying for a PhD degree or in the early phase of a postdoctoral study, with 12 from each side of the Atlantic. Applications can only be accepted from students working in the United States or one of the European Member States and Associated Countries at the time of application, or from students having a citizenship of one of those countries.

Acceptance to the course and for the course fellowship will be made on the basis of application documents and recommendation letters, submitted via the course online system. Submission of applications will be open from **January 1, 2011 until February 28, 2011**. Course participants will be informed by April 4, 2011.

Course organization: Jan Roelof van der Meer, University of Lausanne
Course official website: www.unil.ch/usecbiotech2011.

PRIZES

BODOSSAKI FOUNDATION



BODOSSAKI ARISTEIO PRIZE 2011

In Clinical Medical Research

The Bodossaki Aristeio was instituted to honour scientists of Greek descent whose lifelong track records are internationally recognized for outstanding achievements that contributed greatly to the advancement of their respective fields. The Aristeio is awarded every two years and is accompanied by a prize of €150.000.

The Board of Trustees of the Bodossaki Foundation, at its October 2010 meeting, accepted the recommendation of the International Aristeio Committee that the 2011 Aristeio should be shared equally by two outstanding clinical investigators, namely **Professor Haralampos Moutsopoulos**, of Athens University Medical School for providing significant insights into the pathogenesis of autoimmune systemic rheumatic disorders and **Professor George Chrousos**, of the Athens University Medical School for his pioneering studies on stress pathophysiology and the mechanisms regulating the hypothalamic-pituitary-adrenal axis.

POSITIONS OPEN

FACULTY POSITION in Sustainability (cross-disciplinary) Northwestern University

Northwestern University invites applications for a tenure-track faculty position in sustainability. Sustainability is defined broadly to include energy and natural resource supply and consumption, environmental science and technology, Earth and climate science, and their complex interrelationship with economic, political, and social issues. The successful candidate should have a Ph.D. in a relevant field, and have achieved excellence in research and teaching and recognition in the profession consistent with the rank of Associate or Full Professor in appropriate department(s). The candidate is expected to build a significant program in sustainability that complements other strengths in the McCormick School of Engineering and Applied Sciences and the Weinberg College of Arts and Sciences, and builds bridges to policy study in the College and the Kellogg School of Management. Applicants should submit curriculum vitae, a description of research plans and teaching interests, and the names and contact information of three references to **website: <http://facposition.northwestern.edu/sustainability/>**. The search committee will begin reviewing applications starting December 31, 2010. Questions may be directed to **e-mail: mcc_sustainabilitysearch@northwestern.edu**.

Northwestern University is an Equal Opportunity/Affirmative Action Employer. Qualified women and minorities are encouraged to apply. It is the policy of Northwestern University not to discriminate against any individual on the basis of race, color, religion national origin, sex, sexual orientation, marital status, age, disability, citizenship, veteran status, or other protected group status. Hiring is contingent upon eligibility to work in the United States.

TENURE-TRACK ASSISTANT PROFESSOR Analytical Chemistry Georgetown University

Georgetown University wishes to recruit a tenure-track Assistant Professor in Analytical Chemistry to begin fall 2011. Areas of research are open and should be complementary to existing research directions in the department. Candidates must have a Ph.D. degree in chemistry or a closely related field and postdoctoral training or equivalent is desirable. Development of an internationally recognized research program and teaching at the undergraduate and graduate levels are expected. Please send curriculum vitae, description of research plans, statement of teaching philosophy, and arrange for three letters of recommendation to be sent to: **Faculty Search Committee, Department of Chemistry, Georgetown University, Box 571227, Washington, DC 20057-1227**. For full consideration, complete applications should arrive before January 15, 2011. *Georgetown University is an Affirmative Action/Equal Opportunity Employer; applications from qualified women and minority candidates are encouraged.*

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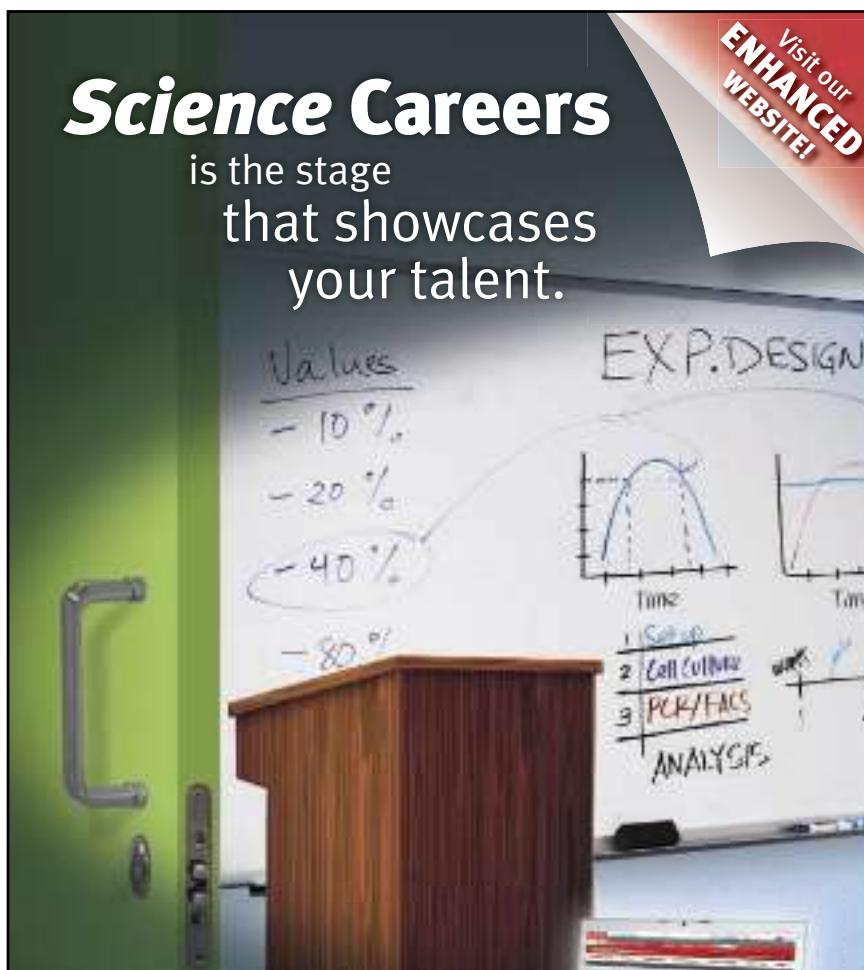
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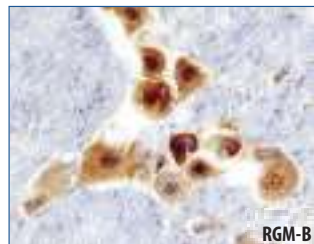
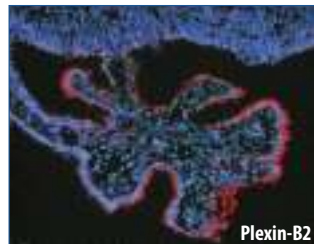
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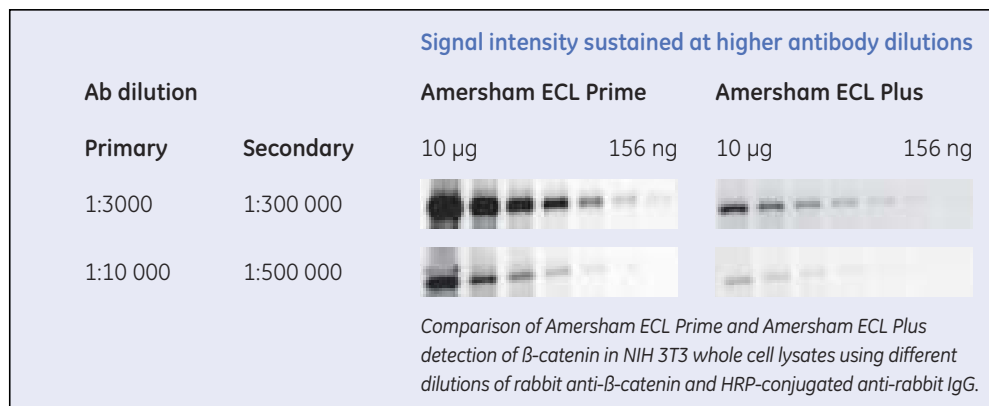
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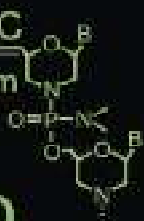
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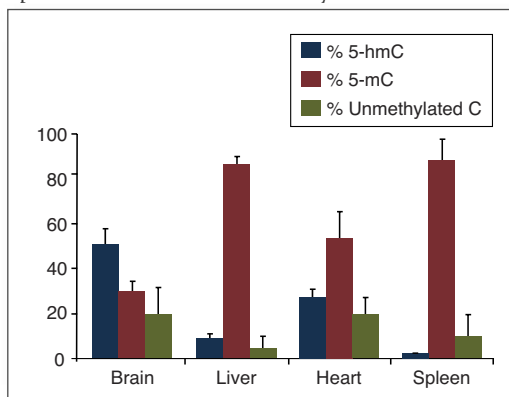


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